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The Chemical Composition and Nutritive Properties of Milk as Affected by the Level of Protein Feeding

Part I. Chemical Composition. A. E. Perkins
Part II. Nutritive Properties. W. E. Krauss and C. C. Hayden

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THE CHEMICAL COMPOSITION AND NUTRITIVE PROPERTIES OF MILK AS AFFECTED BY THE LEVEL OF PROTEIN FEEDING

PART I. CHEMICAL COMPOSITION

A. E. PERKINS[1]

There has been a common and profound belief that the nature of the food supplied to the dairy cow is capable of causing marked changes in the composition or richness of the milk produced. Modern thought tends toward the belief that the quantity of milk produced is much more subject to influence by feeding than is the quality. Quality, as indicated by the proportion of the principal chemical constituents, is more or less definitely established by the breed, individuality, and stage of lactation of the cow, and it is known to be less subject to changes by feeding than was formerly supposed.

Certain aspects of the quality of milk, such as color, flavor, the properties of the fat, the iodine content, and the vitamin content, may be definitely affected by the character of the feed. Sufficient proof is lacking to show whether some of the other important chemical components may or may not be affected by radical differences in the feeding program. Most of the earlier reports of work regarding the effect of different levels of protein feeding considered only the fat and total solids as measures of the composition of the milk. Often the differences in protein content of the rations studied were not striking, and it is doubtful whether the conclusions drawn were justified by the data presented. This may partly account for the diversity of conclusions reached by the early workers.

Recent years have seen great improvement in the application of scientific methods to the control of dairy experimentation; hence, the more recent work, though limited in amount, is probably more reliable than that reported a generation or more ago. The trend of such evidence indicates that the level of protein feeding has com-

[1]The work of many persons has entered into this experiment. The writer is particularly indebted to Mr. C. C. Hayden for his kindly interest, criticisms, and suggestions throughout most of the period; to Mr. R. E. Caldwell, who was in charge of the dairy herd at this Station at the beginning of the experiment; to Messrs. R. I. Grady, C. F. Monroe, and Reynolds Overbeck who, in turn, have assisted with the analytical work; to Messrs. D. V. Strock and William Vordermark and to Miss Emma Collins for much patient work in compiling and tabulating the data.

paratively little influence on the chemical composition of the milk. We have found no record of any previous attempts to study the properties of the fat produced or the proportion of the different proteins present in the milk of dairy cows on different levels of protein feeding.

HISTORICAL

Waters and Hess (33) reported an increase of fat, total solids, and nitrogen in milk produced on rations of about 1:4 N. R.,[2] as compared with that produced under comparable conditions on rations of about 1:6.6 N. R. The observed differences were relatively small and could have been due to other differences in the ration quite as readily as to the protein content.

Lindsey and associates (14) compared rations carrying 1.5, 2.0, and 2.5 pounds of protein, respectively. Some differences were noted between the composition of the milk produced on the wide and narrow rations, but the authors expressed uncertainty that these were due to feeding.

Ingle (12) reported that a protein-rich, as compared with a protein-poor, diet increased both the milk production and the fat content of the milk; whereas freely feeding a wide ration increased the amount of milk but lowered the fat content.

Hayward (10) found from a series of three carefully conducted experiments, based on the method of paired feeding, that variations in the nutritive ratio from 1:3.4 to 1:11.3 had little or no effect on either the quantity or the quality of the milk produced. Hayward withheld publication of his results for some time and studied them most thoroughly because they failed to agree with the prevailing idea of the time and with most of the work published previously.

Crowther (1), continuing the work of Ingle above, observed that changing from a narrow to a wide nutritive ratio increased the milk yield but lowered the fat content, thus seemingly confirming one of Ingle's conclusions with respect to the effect on the composition of the milk. On most points their conclusions are contradictory. On examination of the published reports of these experiments it is evident that the reported differences were mostly within the limits of experimental error and, therefore, are without significance.

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[2]The term "Nutritive Ratio" as used in this publication means the ratio between the digestible crude protein supplied in the ration, indicated in each case as unity, and the carbohydrate equivalent. The latter consists of the sum of the digestible nitrogen-free extract, the digestible crude fiber, and 2.25 times the digestible fat. In other words, it is the ratio between the digestible crude protein and the remaining digestible nutrients.

In this connection also it should be noted that a low-protein ration is often spoken of as a wide ration and a high-protein ration as a narrow ration.

Morgan, Beger, and Fingerling (17), working with sheep and goats, studied the effects of the separate or simultaneous addition of fats and proteins to basal rations containing relatively small amounts of these materials. Both fat and protein are reported to have produced favorable effects on the milk production. Fat had a further specific effect of increasing the fat content of the milk, as well as modifying the character of the fat. However, an increased allowance of protein was without effect either in increasing the proportion or modifying the character of the fat produced.

Morgan, Beger, and Westhausser (18) continued these investigations on sheep and goats, using greater extremes in the level of protein feeding. They found that increasing the level of protein feeding lowered the per cent of fat in the dry matter of the milk and was accompanied by an increase in the percentage of protein and sugar. The milk production was commonly increased by the addition of protein, but the dry-matter content was apparently lowered. Protein feeding was again found to have no perceptible influence on the character of the fat. The character of the fat in this and the preceding experiment was judged solely by the index of refraction.

Lindsey (15) reported the results of an extended series of experiments, finding that ordinary variations in the digestible protein intake exerted little or no effect on the composition of the milk.

Kellner (13), probably the leading European authority of his time on the subject of animal feeds and feeding, in summarizing the older European work reaches this conclusion, as given in the English Edition (1911) of his book.

> "As a rule no influence of the food protein upon the percentage amounts of protein or fat in the milk has been observed. It is only when the body is much reduced in protein that the milk becomes poorer in fat and more watery."

Lindsey gives the following somewhat different translation of this significant passage:

> "In so far as it is possible by means of food to affect the action of the milk-glands, the protein of the several food groups exerts a very pronounced influence. The influence is especially noticeable in increasing the quantity of the milk. Only after the long continued feeding of a ration known to be deficient in protein does the water content of the milk increase and the dry matter and fat show a noticeable decrease." —Translated from Kellner's book, "Die Ernährung der Landwirtschaftliche Nutztiere," 5th Ed. Page 539.

The literature reviewed up to this point represents the status of information concerning this problem at the time this work was undertaken.

Hammond and Hawk (8) and Taylor and Husband (28), working with goats, and Tocher (30), working with Ayrshire cows, have developed the conception that the percentage composition of milk is affected to a much greater degree by the volume of milk produced than by the food of the animal. The proportion of lactose was found by these workers to vary directly as the volume of milk produced; whereas the other ingredients (fat, protein, and ash) were found to vary inversely as the volume.

Taylor and Husband harmonize their findings with the views of Van Der Laan (31) regarding milk secretion, which are, in brief, that milk secretion is largely controlled by osmotic equilibrium between the lactose and mineral matter of the udder secretions and the blood stream. The relation between lactose and mineral matter seems to be of a more or less dual character. The amount of lactose formed from the blood sugar seems to exert a controlling influence on the amount of milk secretion.

Hills and associates (11) concluded from their very extensive work in this field that the total solids and fat content of milk were unaffected by the level of protein feeding employed in their experiments; the levels ranged from approximately 1 pound to 2.5 pounds per day for cows weighing approximately 1000 pounds, or from a nutritive ratio of 1:11.6 to a nutritive ratio of 1:5. The "albuminoid (N x 6.25)" content of the milk seemed to Hills to be slightly influenced (from 1 to 2 per cent) in the direction of the feeding, although this effect was by no means uniform or invariable.

Gowan and Tobey (7) have recently reported work concerning the effect of inanition and of various drugs and hormones on the composition of the milk secreted. Their work confirms, in a general way, the views of Taylor and Husband and those advanced by Van Der Laan which are reviewed above. The writer is unable, however, to harmonize all the details of this hypothesis or that of Taylor and Husband with the performance of dairy cows under normal conditions.

NATURE OF THIS EXPERIMENT

Work designed to study the effects of dairy rations of diverse protein content on the cow and her production was begun at the Ohio Agricultural Experiment Station in 1911 and, with numerous changes in plan, is still in progress. Some of the results have been reported previously (23, 24, 25). Brief summaries also appear in the various annual reports of this Station.

For the first several years of the experiment much emphasis was placed on thorough and systematic chemical analysis of the milk produced on the various rations. These analyses were conducted at frequent and approximately regular intervals. In more recent years the milk analyses have occupied a less conspicuous place in the program. Other features, such as metabolism experiments and analyses of blood and urine from these experimental cows, have taken their place to a considerable extent. Probably the generally negative character of the results of the milk analyses have been chiefly responsible for this change in policy.

THE EXPERIMENTAL ANIMALS

The six cows originally selected from the Station herd for this experiment consisted of one grade Jersey, two purebred Jerseys, two grade Holsteins, and one purebred Holstein. All were young cows of fair productive capacity. Most of them were in early lactation at the beginning of the experiment. All were placed on what was designated as the normal or medium ration having a nutritive ratio between protein and carbohydrate equivalent of 1:6.5 for the remainder of that lactation period.

At the beginning of the succeeding lactation period the cows were divided into three groups of two cows each as nearly equal as possible with respect to breed and productive capacity. One of these groups of two cows was continued indefinitely on the medium ration; another group was transferred to what was known as the wide ration, N. R. 1:9; the third group of two cows was changed to what was then designated as the narrow ration, N. R. 1:4.

The cows in these respective groups were continued for periods of several years, in some cases throughout their productive life, on the respective rations to which they were assigned; and, in accordance with the original plan, the female offspring of the respective groups were reared from weaning time on the same rations received by their dams and continued on these rations after coming into milk. For reasons which probably had no connection with the experiment, the Jersey cows in the groups receiving the wide and narrow rations and the grade Holstein cow in the group receiving the medium ration produced no female progeny. Thus, the Jerseys in two of the groups were automatically eliminated with the passing of the original cows. Because of the absence of any marked difference in the general results obtained between the wide and the narrow rations, the medium ration was later discontinued and both the wide and narrow rations made more extreme in character.

Because there were fewer data from the Jersey cows than from the Holsteins, because these figures were unequally distributed among the various groups, and because of the well known, pronounced differences in composition between Jersey and Holstein milk, the figures from the Jersey cows have been omitted altogether from the comparisons with respect to milk analysis. The breed difference with respect to the character of the fat, however, is less pronounced; hence, the data from the Jersey cows are included among the data used to study the effect of the different rations on the character of the fat produced.

From the standpoint of the data regarding the composition of the milk, it would have been better had the cows been rotated from one ration to another by lactation periods or other suitable intervals, but one of the major purposes of this study was to bring out any possible effect of the long-continued use of protein-rich and of protein-poor rations on the well being of the cow. This purpose could be achieved only by the continuous use of the respective rations. In the later stages of this study some reversals in rations were made. This will be evident from a study of the tables. Such data are also being considered separately in this publication. After a few years, the idea of selecting the experimental animals from progeny produced on the respective rations was abandoned. More recently young purebred Holstein cows of good productive ability, with one lactation period on normal feeding available for comparison, have been the preferred type of animal for use in this experiment.

RATIONS USED

In the early years of this experiment when the great bulk of the data presented in this bulletin were accumulated, three rations having nutritive ratios of 1:4, 1:6.5, and 1:9, respectively, were used continuously on the same cows, as indicated above. The cows were stall-fed throughout the year, but during suitable weather ran in a lot devoid of vegetation. This provision limited the number of cows which could be carried on the experiment but avoided the uncertainty of conclusions drawn from winter feeding experiments only or the uncertain effects of unknown amounts of feed obtained from the pasture. Some of the animals furnishing the data in this experiment completed their entire life cycle without having access to pasture. While this in itself was only incidental to the experiment proper, it was a never failing source of interest and comment on the part of visitors.

The rations were made up of feeds in common use on Ohio farms. For the purpose of avoiding complication of our results with the specific effect of individual feeds, the same feeds were used in all cases in compounding all the original rations. This was done by varying the proportions of the ingredients. Specimens of the three rations of diverse protein content as compounded from the same feeds are shown herewith. A detail showing the adaptation of one of these rations for the guidance of the feeder and another showing the method used in calculating the rations are also presented.

Rations of Diverse Protein Content from the Same Feeds

Feed used	Narrow Ration N. R. 1 : 4	Medium Ration N. R. 1 : 6.5	Wide Ration N. R. 1 : 9
	Lb.	*Lb.*	*Lb.*
Alfalfa Hay	9.0	6.0	3.25
Timothy Hay	3.0	4.0	6.50
Silage	15.0	30.0	32.50
Corn	2.25	4.8	6.50
Bran	2.25	0.9	1.45
Cottonseed Meal	1.8	0.9	0.28
Linseed Oilmeal	1.8	0.9	0.29
Total Hay in above	12.0	10.0	9.75
Total Grain in above	8.1	7.5	8.50
Total Silage in above	15.0	30.0	32.50

Digestible Nutrients in Feeds (1 lb. Contains)

	Protein	Carbohydrate equivalent
Alfalfa Hay	.115	.368
Timothy Hay	.024	.424
Silage	.011	.175
Corn	.0597	.690
Bran	.1124	.468
Cottonseed Meal	.330	.389
Oilmeal	.336	.438

Detail Showing Calculation of a Ration

	Digestible crude protein		Carbohydrate equivalent, equals digestible crude fiber plus digestible nitrogen-free extract, plus 2.25 x digestible fat
2 alfalfa hay	.230		.736
⅔ timothy hay	.016		.282
3⅓ silage	.037		.578
.5 corn	.030		.345
.4 cottonseed meal	.130		.154
.5 bran	.056		.234
.4 oilmeal	.133		.174
	.632		2.503
Nutritive ratio	1	:	3.96

Detail Showing the Adaptation of a Ration for the Convenience of the Feeder

Alfalfa Hay	1.5	2	3	4	5	6	7	8	9
Timothy Hay	0.5	0.67	1	1.33	1.66	2	2.33	2.66	3
Silage	2.5	3.33	5	6.67	8.33	10	11.67	13.33	15
Corn	0.38	0.5	0.75	1.0	1.25	1.5	1.75	2.00	2.25
Bran	0.38	0.5	0.75	1.0	1.25	1.5	1.75	2.00	2.25
Cottonseed Meal	0.3	0.4	0.6	0.8	1.00	1.2	1.40	1.60	1.80
Oilmeal	0.3	0.4	0.6	0.8	1.00	1.2	1.40	1.60	1.80
Total Hay	2	2.67	4	5.33	6.66	8.0	9.33	10.66	12
Total Grain	1.36	1.80	2.7	3.60	4.50	5.40	6.30	7.20	8.1

For the most part, the rations were calculated from our own actual analyses of the feeds used and from average digestion coefficients. At times, however, the tables of average analyses published by Henry and Morrison were used to supplement our own analyses.

The general feeding instructions for the experiment specified that the animals were to receive as much of the respective rations at all times as they would consume without undue waste. If it became necessary to change the amount of one of the ingredients to meet the apparent needs of the cow or to avoid excessive waste of feed, all ingredients of the ration were changed in the same proportion.

The grain portion of the ration was fed in the form of a mixture, and all the feed supplied the cows was regularly weighed and recorded. Maintaining an arbitrary proportion between the hay, grain, and silage as described (which seemed necessary to keep the protein in the prescribed proportion) resulted in some undesirable features. The level of grain feeding in early lactation was limited by the cow's ability to consume the corresponding amount of roughage. On this account also, more grain was fed during the stripper and dry periods than would be supplied in ordinary good feeding practice. Another drawback was that one unpalatable ingredient tended to restrict the consumption of the entire ration and thus probably had, in many instances, a depressing effect on the production. The timothy hay included in each of these rations in varying amounts was noteworthy in this respect, since it was the least palatable ingredient of the rations. At times, also, some of the high-protein grain mixtures seemed to become distasteful to the cows. In the later work, in which greater contrasts in feeding were used, no attempt was made to include all the same ingredients in each ration of opposite extreme, but a good variety of feeds was employed in all cases to minimize the danger of the possibly excessive influence of individual feeds.

SPECIMEN RATION N. R. 1:13

Feed	Daily allowance, pounds
Timothy Hay	10
Corn Silage	30
Cane Molasses	1.5 (on the hay)
Grain Mixture	10

TYPICAL GRAIN MIXTURE, 1:13 RATION

Corn	600 lb.
Oats	100 lb.
Bran	100 lb.
Corn Starch	100 lb.

SPECIMEN RATION N. R. 1:11

Feed	Daily allowance, pounds
Timothy	10.5
Sugar Beet Pulp, dry weight	9.8
Corn	6.5
Oats	3.25
Bran	3.25
Cane Molasses	1.3 (on the hay)

SPECIMEN RATION N. R. 1:2

Feed	Daily allowance, pounds
Alfalfa Hay	16
Corn Silage	16
Grain Mixture	12

TYPICAL GRAIN MIXTURE, 1:2 RATION

Corn Gluten Meal	100 lb.
Cottonseed Meal	100 lb.
Linseed Oilmeal	100 lb.
Soybean Oilmeal	100 lb.
Peanut Oilmeal	100 lb.
Wheat Bran	100 lb.
Blood Meal	150 lb.
Wheat Gluten	150 lb.

Typical examples of the 1:2, 1:11, and 1:13 rations used in the later stages of the experiment are also given herewith. The rations given as typical were not rigidly and invariably adhered to. The experiment was concerned with the proportion of protein in the rations and not with the effect of individual feeds. Sometimes clover hay or soybean hay was used in place of alfalfa hay, as given in the sample rations, soaked dried sugar beet pulp and, in one or two instances, some green corn were substituted for the corn silage when the latter was no longer available. Oat straw or soybean straw has been substituted for timothy hay on one or two occasions when no timothy hay was procurable. The rations were recalculated and revised whenever necessary to meet such conditions, and it is felt that the prescribed proportion between the ingredients of the ration was on the whole satisfactorily maintained.

WEIGHING AND SAMPLING THE MILK

The cows in this experiment were milked twice daily.

The weight of milk produced at each milking was ascertained and recorded. The samples of milk on which the analyses constituting the data of this bulletin are based were taken in the following manner. The entire amount of milk produced by each of the cows on the experiment at the evening milking of the designated day was placed in a separate tinware can, tightly covered, and kept in a cold room until the following morning. The entire morning milking from each cow was likewise saved in a separate, covered container. The morning and the evening milk from each cow were combined and thoroughly mixed by repeated pouring, and a subsample of suitable size was removed for the detailed analysis of the milk. The bulk of the milk was then either used for the preparation of the fat sample or returned to the general milk supply.

Samples of butterfat for analysis were also prepared from this milk; the residue of the milk sampled as described above was run through a centrifugal separator, the cream was churned, and the resulting butter washed and then melted and kept in a molten but not overheated condition to allow the separation of the fat from the adhering curd and water. The water was removed from the bottom of the container with a siphon or pipette. The fat, still maintained in a molten condition, was then filtered through a dry paper filter. The fat so prepared was kept in glass-stoppered bottles in a dark, cold room until the chemical analyses were about to be made.

Each sample of milk or fat so prepared fairly represented the production of one day. The milk analyses were carried out on the fresh samples without the use of preservatives, a point of no small importance in view of the alteration in the composition of milk which may be brought about by the use of preservatives, as shown by the writer's unpublished experience in making fat tests and protein separations and also as described by Palmer and Coolidge (19).

Samples for the proximate, or partial, analyses of the milk, including the determinations of fat, total solids, total protein, sugar, and ash, were taken at intervals of 2 or 3 weeks. Samples of fat were prepared only one-half as often, and a more complete analysis of the milk, including a separate determination of the various proteins, was carried out on the same occasions when the fat samples were taken. All stages of the lactation period are equally represented in the analyses thus obtained, except that very few samples were taken during the 5-day period following parturition when the milk has more or less of the characteristics of the colostrum, or first-milk.

METHODS OF ANALYSIS

MILK

The methods of analysis employed were, for the most part, those officially or provisionally recommended by the Association of Official Agricultural Chemists[3] (29), although there are important exceptions to this general rule.

Milk samples for the determination of total solids, total nitrogen, casein, ash, and lactose were measured by the use of special pipettes designed by the writer (21) to compensate for the differences in specific gravity of the milk and its tendency to adhere to the pipette during measurement. The specific gravity of the milk was determined by means of a very sensitive lactometer described by Shaw and Eckles (27) and now listed regularly by some dealers in chemical laboratory supplies.

Total solids were determined on 10-gram samples of milk measured into previously weighed, flat-bottomed, porcelain or vitreosil dishes. The samples were then dried to approximately constant weight at the boiling temperature of water.

Ash was determined by the ignition of the dry residue from the solids determination at the lowest practicable temperature. At first, this was done over an open gas flame and more recently in an electrically-heated muffle.

Nitrogen was determined by one of the recognized modifications of the Kjeldahl method, control blank determinations being made frequently. In harmony with the practice of biochemists, the factor 6.38 was used to convert nitrogen to total protein, casein, or albumin, as the case might be.

Fat was determined by the Babcock volumetric method.

Lactose was determined by means of a half-shadow polariscope equipped with a direct reading Ventzke sugar scale. The details of the method employed varied from those recommended by the A. O. A. C. Instead of an arbitrary correction for volume of precipitate, the proper correction is calculated for each sample, as described by the writer (22).

Casein was precipitated essentially by the A. O. A. C. method by diluting 10 grams of milk to about 100 cc. with water at about 40 to 45 degrees C. in a 250 cc. beaker and adding 15 cc. of 1 per cent acetic acid, accompanied by vigorous and repeated stirring. The beakers were covered and allowed to stand over night. The precipitate was washed twice by decantation, then collected on a paper filter, the beaker and stirring rod being thoroughly cleaned with a

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[3]Hereafter, for the sake of brevity, this is referred to as A. O. A. C.

rubber policeman. The casein was then thoroughly washed on the filter. The grade of filter used is of especial importance in securing satisfactory results with this determination. The author prefers the Munktell's O. B. or O. O. grade, although some other papers of similar texture may be satisfactory.

Albumin was at first determined by the method, introduced by Van Slyke and adopted by the A. O. A. C. (29, 32), of heat precipitation of the filtrate from the casein determination after adjustment to proper acidity. Since it was realized that this was not comparable with the determination of albumin in milk as developed by Sebelien (26) and other German chemists and later adopted by biochemists in this country (9), the filtrate from this determination of heat-coagulable albumin by the A. O. A. C. method has been regularly precipitated by the addition of 15 cc. of Almen's reagent. The heat-coagulable albumin and the albumin precipitated later from the same solution by the Almen's tannic acid reagent were determined and recorded separately. The heat precipitation of the albumin by the A. O. A. C. method often gave much difficulty. It was found that the addition of a few grams of some neutral salt, such as potassium or sodium sulfate, greatly increased the speed and completeness of precipitation. The addition of either of these salts likewise improved the coagulation of the tannic acid precipitate. The values as determined separately for either the heat-coagulable albumin or the tannic acid precipitate seemed erratic and unreliable, but the sum of the two seemed more consistent in its variations. In the present discussion the sum alone is considered, being treated as a single value. In the later phases of this work the tannic acid precipitation has been applied directly to the filtrate from the casein determination, with the addition of potassium sulfate. The separate determination of the heat-coagulable portion of the albumin has been omitted.

ANALYSIS OF THE FATS

As is well known to chemists, no practical methods are available for separating and determining directly the individual component fats of a natural mixture of fats such as butter. The best that can be done is to resort to the determination of certain empirical values known as physical and chemical constants, which reveal, although roughly and indirectly, the composition of the fat being examined. A great variety of different determinations is available for this purpose. Those selected and used to reveal the character of the fat produced in this experiment were: The saponification value, which indicates indirectly the mean molecular weight of the fats present; the Reichert-Meissl value, which is a measure of the volatile fatty acids, the most characteristic feature distinguishing butter from other fats; the iodine value, which indicates the proportion of unsaturated fatty acids; the hardness, which shows the resistance offered by the fat to mechanical force applied in attempting to change its form. The hardness of a sample of butter or butterfat is very greatly influenced by the temperature at the time of testing and also by the temperature at which the sample has been held for several hours just preceding the test. Considerable discussion is to be found in the literature regarding the effect of different feeds on the hardness of the fat produced, the hardness of butter having a widely recognized commercial importance. It is to be feared that all the older work is practically meaningless, however, because of the lack of a suitable and sufficiently sensitive method of measuring the hardness. In this work a method originated and described by the writer (20) has been used. While still not without its faults and difficulties, the method is certainly far more accurate and appropriate than any other which has been proposed.

The first three of these determinations were made according to methods recommended by the Association of Official Agricultural Chemists. The Hanus modification of the iodine value determination and the Leffman and Beam modification of the Reichert-Meissl value determination were followed.

Doubtless other determinations would have been desirable, but practical considerations of time and equipment seemed to set the limit at this point, and it is felt that the range of determinations which were carried out was sufficient to detect any major variation which might have occurred under the influence of the feeding program described.

PRESENTATION OF RESULTS

Rather complete analyses of more than 1000 samples of milk are represented in this work. The presentation in detail of so many figures is hardly to be thought of, especially in view of the generally negative character of the results. In Tables 1 to 3, there are presented, however, specimens of these detailed figures for one cow during one period of lactation to illustrate the method followed in deriving the results presented in the later tables. Table 1, which is largely self-explanatory, contains the necessary information about the samples, the amount of milk produced, and the various analyses stated as per cents of the sample analyzed.

TABLE 1.—Specimen of Analysis of Milk. Cow 111. First Period. Fresh, April 27, 1915

Laboratory No.	Date 1915-1916	Specific gravity	Total solids	Fat	Lactose	Ash	Nitrogen occurring as: Total	Casein	Heat coagulable	Tannic acid precipitate	Residual	Milk production (daily)
			Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Lb.*
364	5/3	1.0321	14.48	4.90	4.14	.801	.609	.448	.069	.044	.048	26.6
370	5/24	1.0307	11.86	3.70	5.19	.694	.424	....	....	....	....	26.0
377	6/15	1.0302	12.02	3.80	5.20	.643	.394	.301	.043	.030	.020	28.5
382	7/7	1.0311	11.30	3.15	4.98	.693	.394	....	....	....	....	24.4
388	7/26	1.0313	11.08	2.80	4.67	.652	.406	.316	.042	.034	.014	27.2
393	8/17	1.0308	11.43	2.80	4.79	.661	.397	....	....	....	....	23.5
397	9/6	1.0311	11.71	3.30	4.96	.641	.415	.314	.033	.052	.016	24.2
400	10/1	1.0312	11.72	3.10	4.96	.660	.438	....	....	....	....	19.2
405	10/21	1.0322	11.42	2.90	5.09	.710	.450	.350	.041	.030	.029	22.7
409	11/16	1.0315	11.92	3.15	4.78	.702	.450	....	....	....	....	18.5
414	12/6	1.0318	12.06	3.60	5.10	.693	.427	.342	.042	.028	.015	21.5
419	12/28	1.0329	12.81	3.25	5.42	.687	.457	....	....	....	....	20.3
424	1/18	1.0333	12.28	3.40	5.43	.674	.469	.371	.044	.031	.026	18.3
429	2/8	1.0327	12.38	3.50	5.26	.667	.493	....	....	....	....	17.8
434	2/28	1.0338	12.11	3.40	4.89	.676	.494	.400	.043	.033	.018	17.2
440	3/20	1.0357	14.28	4.30	5.12	.757	.625	....	....	....	....	10.4
446	4/11	1.0351	13.70	4.00	5.29	.729	.598	.486	.059	.034	.019	10.6
452	5/2	1.0380	14.94	4.10	5.21	.816	.721	....	....	....	....	7.2
Total	....	....	223.50	64.15	90.48	12.556	8.661	3.328	0.416	0.316	0.205	....
Direct average, per cent	....	....	12.42	3.56	5.03	0.698	0.481	0.370	$\underbrace{0.046 \quad 0.035}_{0.081}$		0.023	....

TABLE 2.—Specimen Showing Method of Deriving Weighted Average Analyses. Cow 111. First Period

Laboratory No.	Production in pounds										
	Milk	Solids	Fat	Protein	Lactose	Ash	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Milk
364	26.6	3.852	1.303	1.034	1.101	.213	.16200	.11917	.03006	.01277	26.6
370	26.0	3.084	0.962	0.703	1.349	.180	...	...	...	...	...
377	28.5	3.426	1.083	0.717	1.482	.183	.11230	.08579	.02081	.00570	28.5
382	24.4	2.757	0.769	0.613	1.215	.169	...	...	...	...	...
388	27.2	3.014	0.762	0.704	1.270	.177	.11043	.08595	.02067	.00381	27.2
393	23.5	2.686	0.658	0.595	1.126	.155	...	...	...	...	...
397	24.2	2.834	0.797	0.641	1.200	.155	.10043	.07599	.02057	.00387	24.2
400	19.2	2.250	0.595	0.537	0.952	.127	...	...	...	...	...
405	22.7	2.592	0.658	0.652	1.155	.161	.10215	.07945	.01612	.00658	22.7
409	18.5	2.205	0.583	0.532	0.884	.130	...	...	...	...	...
414	21.5	2.593	0.774	0.586	1.097	.149	.09181	.07353	.01505	.00323	21.5
419	20.3	2.600	0.660	0.592	1.100	.139	...	...	...	...	...
424	18.3	2.247	0.622	0.547	0.994	.123	.08583	.06789	.01318	.00476	18.3
429	17.8	2.204	0.623	0.560	0.936	.119	...	...	...	...	...
434	17.2	2.083	0.585	0.542	0.841	.116	.08497	.06880	.01307	.00310	17.2
440	10.4	1.485	0.447	0.415	0.532	.079	...	...	...	...	...
446	10.6	1.452	0.424	0.405	0.561	.077	.06339	.05152	.00986	.00201	10.6
452	7.2	1.076	0.295	0.331	0.375	.059	...	...	...	...	...
Total	364.1	44.440	12.602	10.706	18.170	2.511	0.91331	0.70809	0.15939	0.04583	196.8
Weighted average production	20.23	2.469	0.700	0.595	1.009	0.1395	0.10148	0.07867	0.01771	0.00509	...
Weighted average, per cent	...	12.20	3.46	2.94	4.99	0.690	0.464	0.360	0.081	0.023	...

Table 2 shows the amount, in pounds, of the various constituents of the milk produced on the day when the sample was taken, found by multiplying the per cent of each ingredient by the amount, in pounds, of milk produced on that day. The total production of each ingredient on all the days of sampling divided by the total amount of milk production on these days gives the true or weighted average percentage composition of the milk on these days, which are uniformly distributed throughout the lactation period. This is practically the same result which would be obtained if it were practicable to collect and analyze a composite sample representing the entire lactation period. The various values will probably be somewhat different from those obtained by a direct average of the figures representing the analyses at different times, for the composition of the larger amounts of milk produced near the beginning of lactation is often quite different from that of the small amounts obtained near the close of lactation. In the direct average, each of these analyses is given equal weight. In the true or weighted average, the former may exert several times as much influence as the latter on the final result because of the greater amount of milk represented (Last lines of Tables 1 and 2).

In the case of the chemical constants of the fat, as shown in Table 3, a somewhat different explanation is needed, although the process and results are much the same as those just given for the milk. Being abstract values or properties of the butterfat, these values must be thought of as being multiplied or weighted by the amount of fat produced on that occasion as a factor and not as the production of definite amounts of some substance. The reason for using the weighted average rather than the direct average values is the same in both cases.

RESULTS WITH THE ORIGINAL WIDE, MEDIUM, AND NARROW RATIONS

In Tables 4, 5, and 6 are shown the results of the milk analyses for the 1:9, 1:6.5, and 1:4 rations, respectively, arranged to present the weighted average values for the different determinations for each lactation period of each cow. The results are grouped and averaged according to the ration received by the cows, as indicated in the table headings. The results are brought together for comparison in Table 7. If the results from the 1:4 and 1:9 rations stood alone, it would seem justifiable to attribute an increase of approximately 0.5 per cent in solids, 0.16 per cent in fat, 0.29 per cent in total nitrogen, and lesser changes in some of the other

TABLE 3.—Specimen Showing Method of Deriving Weighted Average Results for Chemical Constants of Butterfat. Cow 111. First Period

Laboratory No.	Milk production	Fat	Fat production	Chemical constants as determined				Weighted constants (constants x fat production)			
				Saponification value	Reichert-Meissl value	Iodine value (Hanus)	Hardness at 10° C.	Saponification value	Reichert-Meissl value	Iodine value (Hanus)	Hardness at 10° C.
	Lb.	*Pct.*	*Lb.*								
364	26.6	4.9	1.303	224.0	27.2	42.8	0.67	291.872	35.442	55.768	0.873
377	28.5	3.8	1.083	217.5	21.9	46.2	0.46	235.553	23.718	50.035	0.498
388	27.2	2.8	0.762	226.0	27.4	38.2	1.07	172.212	20.879	29.108	0.815
397	24.2	3.3	0.799	227.2	26.2	37.6	1.62	181.533	20.934	30.042	1.294
405	22.7	2.9	0.658	230.6	28.8	36.5	0.98	151.735	18.950	24.017	0.645
414	21.5	3.6	0.774	230.6	28.7	32.4	2.00	178.484	22.214	25.078	1.548
424	18.3	3.4	0.622	227.4	27.2	35.2	1.43	141.443	16.918	21.894	0.889
434	17.2	3.4	0.585	226.8	27.4	37.4	0.64	132.678	16.029	21.879	0.374
446	10.6	4.0	0.424	222.0	24.0	38.2	0.83	94.128	10.176	16.197	0.352
Total			7.010	2032.1	238.8	344.5	9.70	1579.638	185.260	274.018	7.288
Direct average				225.8	26.5	38.3	1.08	Weighted average values			
								225.3	26.4	39.1	1.04

TABLE 4.—Composition of Milk Produced on Low-protein (1:9) Ration, in Per Cent

Cow	Period	No.* samples analyzed	Solids	Fat	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Lactose	Ash	Milk production in pounds
59	2nd	7	11.53	3.09	.445	.335	.086	.024	4.75	.662	26.3
	3rd	7	11.04	2.99	.420	.309	.095	.016	5.20	.707	22.6
	4th	9	11.24	3.06	.447	.328	.099	.020	4.84	.680	19.2
	5th	8	11.54	3.14	.439	.335	.086	.018	4.84	.662	19.9
	6th	10	11.48	3.00	.442	.320	.096	.026	4.38	.677	18.9
111	1st	9	12.20	3.46	.461	.359	.081	.021	4.99	.690	21.9
	2nd	10	11.94	3.08	.470	.365	.079	.026	4.74	.678	24.3
	3rd	8	11.72	3.09	.455	.354	.073	.028	5.03	.693	29.4
	4th	4	12.14	3.21	.456	.361	.076	.019	5.06	.723	29.4
	5th	4	12.12	3.42	.446	.344	.075	.026	4.96	.723	29.6
	6th	5	12.02	3.27	.438	.330	.086	.021	4.95	.681	27.7
154	1st	7	12.13	3.56	.466	.355	.090	.022	4.87	.684	21.7
	2nd	4	12.17	3.49	.479	.366	.085	.028	4.97	.706	28.6
	3rd	4	11.91	3.74	.449	.343	.082	.025	4.92	.699	30.3
	4th	5	11.84	3.30	.428	.324	.083	.020	5.05	.661	31.5
146	5th	4	12.63	3.85	.464	.348	.099	.017	4.82	.753	36.0
Total			189.65	52.70	7.205	5.476	1.371	0.357	78.37	11.079	417.3
Average			11.85 ±0.066†	3.29 ±0.044	0.450 ±0.003	0.342 ±0.003	0.086 ±0.001	0.022 ±0.001	4.90 ±0.030	0.692 ±0.004	26.08

*The number of samples shown in this column indicates the number on which the protein separations were carried out. The total of samples analyzed was approximately double this number.

†The values for probable error were calculated by using the lactation average values in these tables as though they were single observations.

TABLE 5.—Composition of Milk Produced on Medium-protein (1:6.5) Ration, in Per Cent

Cow	Period	No.* samples analyzed	Solids	Fat	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Lactose	Ash	Milk production in pounds
59	1st	13	11.42	3.03	.483	.365	.088	.030	4.83	.670	14.7
66	1st	21	12.59	3.58	.501	.391	.084	.026	5.07	.707	25.8
67	1st	19	13.21	3.97	.520	.421	.074	.024	5.14	.725	19.7
	2nd	13	12.98	4.05	.509	.411	.073	.025	4.70	.728	23.9
	3rd	14	13.06	4.25	.482	.378	.078	.026	4.76	.679	20.6
	4th	16	12.82	4.04	.513	.407	.080	.026	4.92	.716	22.1
170	1st	11	12.41	3.66	.475	.379	.076	.019	4.91	.722	21.5
	2nd	10	12.25	3.62	.481	.378	.079	.024	4.76	.717	19.0
	3rd	9	12.23	3.60	.475	.369	.086	.021	5.03	.685	27.6
59	7th	13	11.31	3.07	.439	.323	.087	.029	4.72	.698	19.3
Total			124.28	36.87	4.878	3.822	0.805	0.250	48.84	7.047	214.2
Average			12.43 ±0.130†	3.69 ±0.080	0.488 ±0.005	0.382 ±0.006	0.081 ±0.002	0.025 ±0.0006	4.88 ±0.030	0.705 ±0.004	21.42

*See note Table 4.
†See note Table 4.

TABLE 6.—Composition of Milk Produced on High-protein (1:4) Ration, in Per Cent

Cow	Period	No.* samples analyzed	Solids	Fat	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Lactose	Ash	Milk production in pounds
66	2nd	12	12.46	3.97	.486	.362	.089	.035	4.79	.690	24.5
	3rd	6	12.61	3.88	.512	.394	.090	.028	4.86	.731	18.1
	4th	12	12.13	3.85	.465	.337	.091	.037	4.65	.682	23.9
	5th	13	12.18	3.68	.460	.335	.089	.036	4.73	.711	28.5
	6th	15	12.34	3.74	.461	.336	.090	.035	4.78	.733	19.8
146	1st	7	12.45	3.74	.488	.365	.083	.040	4.81	.725	27.8
	2nd	4	12.23	3.56	.467	.354	.082	.031	4.87	.727	34.3
	3rd	5	12.35	3.98	.505	.379	.090	.036	4.54	.749	32.1
	4th	6	12.56	4.08	.458	.325	.098	.035	4.78	.709	28.6
111	7th	8	11.92	3.40	.456	.333	.096	.028	4.97	.705	31.5
192	1st	6	12.36	3.73	.500	.373	.096	.032	4.76	.728	22.2
	2nd	5	12.53	3.84	.487	.351	.095	.041	4.87	.675	29.8
Total			148.12	41.45	5.745	4.144	1.089	0.414	57.41	8.555	321.1
Average			12.34 ±0.037†	3.45 ±0.035	0.479 ±0.004	0.345 ±0.004	0.091 ±0.001	0.035 ±0.001	4.78 ±0.021	0.713 ±0.004	26.76

*See note Table 4.
†See note Table 4.

TABLE 7.—Percentage of Constituents Present in Milk

	Average daily milk production	Solids	Fat	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Lactose	Ash
Low-protein (1:9) Ration	26.08	11.85 ±0.066*	3.29 ±0.044	0.450 ±0.003	0.342 ±0.003	0.086 ±0.001	0.022 ±0.001	4.80 ±0.030	0.692 ±0.004
Medium-protein (1:6.5) Ration	21.42	12.43 ±0.130	3.69 ±0.080	0.488 ±0.005	0.382 ±0.006	0.081 ±0.002	0.025 ±0.0006	4.88 ±0.030	0.705 ±0.004
High-protein (1:4) Ration	26.76	12.43 ±0.037*	3.45 ±0.035	0.479 ±0.004	0.345 ±0.004	0.091 ±0.001	0.035 ±0.001	4.78 ±0.021	0.713 ±0.004

*The values for probable error were calculated by using the lactation average values in Tables 4 to 6 as though they were single observations.

ingredients to the difference in protein content of the rations. When the figures for the 1:6.5 ration are also included in the comparison, however, it is seen at once that there is no regular progression of these values from the lower to the higher levels of protein feeding. Individuality of the cows or other causes clearly outrank the level of protein feeding insofar as any effect on the amount of the principal ingredients of the milk is concerned. Attention is called to the average amount of milk secretion on the medium ration, which is approximately 5 pounds per day less than on either of the other rations. The increases in percentage of total solids and of fat and total nitrogen which accompany this decreased volume of milk appear to bear out the views advanced by Taylor and Husband and others that the composition of milk is influenced to a greater extent by the volume of milk secreted than by changes in the composition of the food. In the case of one ingredient, the residual or non-protein nitrogen, there is seen to be a regular and progressive increase accompanying the increase in the level of protein feeding to which attention will be called in considering the remaining data to be presented.

RESULTS FROM THE SAME COWS ON DIFFERENT RATIONS

Although reversal of rations was not practiced in this experiment to any great extent, some of the cows after several lactations on one ration were given rations of decidedly different protein content. The data regarding the composition of the milk under such conditions are presented in Table 8. Here it is apparent that the average values for total solids and fat are in remarkably close agreement. Although there seem, at first glance, to be noteworthy differences between the two groups in the case of total nitrogen, casein nitrogen, albumin nitrogen, and possibly also in the case of the lactose, none of these differences will stand the tests for statistical significance which are usually considered to apply to data of corresponding type and amount. Here again, however, the amount of residual or non-protein nitrogen contained in the milk from the same cows on different levels of protein feeding was decidedly higher in the case of the high-protein rations.

TABLE 8.—Composition of Milk from Same Cows in Different Periods Using Different Rations

Per cent of various ingredients

Cow	Nutritive ratio of ration	Period	No.* samples analyzed	Total solids	Fat	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Lactose	Ash	Average daily milk production in pounds
Wide Rations												
111	1:9	Av. 6	40	12.02	3.25	.454	.352	.078	.023	4.95	.698	27.1
146	1:9	5th	4	12.63	3.85	.464	.348	.099	.017	4.82	.753	36.0
154	1:9	Av. 4	20	12.01	3.52	.455	.347	.085	.024	4.95	.687	28.0
154	1:11	Av. 2	7	11.68	3.18	.436	.316	.096	.021	5.04	.689	32.7
Total			71									
Average				12.09	3.45	0.452	0.341	0.090	0.021	4.94	0.707	30.9
Narrow Rations												
111	1:4	7th	8	11.94	3.40	.456	.333	.096	.028	4.97	.705	31.5
146	1:4	Av. 4	22	12.40	3.84	.479	.356	.088	.036	4.75	.727	30.7
154	1:2	Av. 2	13	11.61	3.13	.496	.360	.103	.033	4.61	.710	17.8
Total			43									
Average				11.98	3.46	0.477	0.350	0.096	0.032	4.79	0.714	26.6

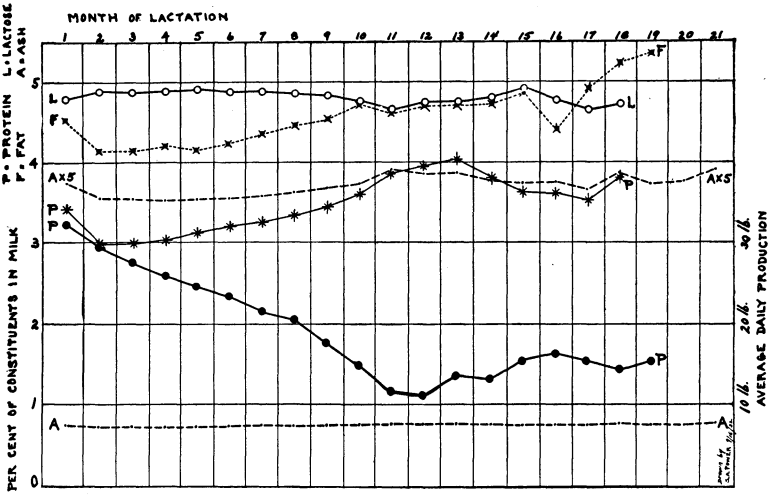
*See note Table 4.

RESULTS WITH EXTREME RATIONS

A smaller number of analyses is available on milk produced by cows receiving rations of 1:2 and 1:13 nutritive ratio, respectively. This is as wide a difference in protein content of the ration as can be practically maintained over long periods without introducing other abnormal conditions. The cows of one group were at times literally starving for protein, although getting all they would eat of the prescribed ration; the others were often receiving 3 or 4 times as much protein as needed, according to accepted standards. Surely, if the protein content of the food exerts any appreciable influence on the composition of the milk, such influences should be strikingly manifest in the milk produced under such extremes of protein feeding as this. The fact that no noteworthy differences in the feeding value of the milk were observed in the work recorded in Part II of this publication serves as further confirmation of these negative results obtained in Part I. The results are shown in Tables 9 and 10. The average values for total solids, fat, total nitrogen, casein nitrogen, and lactose of the milk are all somewhat higher in the case of the 1:13 ration.

We have been at a loss to know what rules of significance should be applied to these, as well as to the preceding, data. The differences in the composition of the milk which might be ascribed to the extreme differences in the levels of protein feeding, however, are far less than those which commonly occur in the milk of the same cow on uniform feeding at different times in the lactation period. This is shown by a suitable arrangement of the present data which may be seen in Graph 1, or as shown by the work of Eckles and Shaw (3). The differences are also less than those which are common between different cows under identical conditions or in the same individual under different conditions, as may be seen from the data in Tables 4, 5, and 6, or from the work of Eckles and Shaw (4, 5).

It does seem significant, however, that most of the differences in the composition of milk which workers in the past have ascribed to differences in protein feeding have been in an opposite direction to those shown by the results in Tables 9 and 10. When the amount of the average daily production in each case is taken into account, we are again reminded of the conclusion of Taylor and Husband (28) and of Tocher (30) that the amount of production had a greater influence than did the character of the food in determining the percentage composition of the milk. In this case, however, the decreased amount of production was clearly due to the low level of protein in the ration.



Graph 1.—Showing the effect of advancing lactation on total production and on the proportion of the ingredients of milk

P*=Protein content P°=Production

THE RESIDUAL OR NON-PROTEIN NITROGEN

With respect to the residual or non-protein nitrogen, it is seen to be practically twice as much in the case of the 1:2 ration as in the case of the 1:13 ration. In every comparison it has been significantly higher in the milk of the cows receiving the high-protein rations. Taylor and Husband (28) noted an increase in non-protein N as the result of high-protein feeding. They refer to this material as being "In the milk but not of it".

In addition to determining the residual or non-protein nitrogen by difference, as has been done regularly, we have in a few instances determined directly by a modification of the Kjeldahl method the nitrogen content of the filtrate remaining after the removal of the material precipitated by tannic acid in the usual process of analysis. A few such results are shown in Table 11. In a general way, these results agree closely with those obtained by difference and presented in the other tables.

Denis and Minot (2) developed methods for determining several of the non-protein substances found in blood and milk and report determinations made on the milk of cows in commercial dairies receiving different amounts of protein in the ration.

TABLE 9.—Constituents of Milk Produced on Extremely Low Protein (1:13) Ration

Per cent of various ingredients

Cow	No.* samples analyzed	Total solids	Fat	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Lactose	Ash	Average daily milk production in pounds
264	13	11.70	3.41	.447	.341	.091	.015	4.62	.679	15.9
293	8	12.17	3.42	.531	.404	.097	.030	4.86	.691	12.3
301	3	12.94	3.87	.513	.401	.093	.019	5.02	.697	21.2
	3	12.71	3.64	.490	.393	.089	.008	5.19	.634	16.6
230	3	11.79	3.18	.443	.341	.072	.030	5.14	.684	18.7
Total	30	61.31	17.52	2.424	1.880	0.442	0.102	24.83	3.395	84.3
Average	....	12.26	3.50	0.485	0.376	0.088	0.020	4.97	0.679	16.9

*See note Table 4.

TABLE 10.—Constituents of Milk Produced on Extremely High Protein (1:2) Ration

Per cent of various ingredients

Cow	No.* samples analyzed	Total solids	Fat	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Lactose	Ash	Average daily milk production in pounds
154	13	11.61	3.13	.496	.360	.103	.033	4.66	.710	17.9
292	11	12.45	3.68	.489	.352	.098	.038	4.89	.670	29.9
329	6	12.03	2.85	.451	.307	.099	.046	4.40	.790	26.2
332	5	10.84	3.23	.434	.313	.083	.039	4.90	.706	25.5
Total	35	46.93	12.89	1.871	1.332	0.383	0.156	18.85	2.876	99.5
Average	....	11.73	3.16	0.468	0.333	0.096	0.039	4.71	0.719	24.9

*See note Table 4.

TABLE 11.—Residual or Non-protein Nitrogen in Milk

Direct determination by Kjeldahl method on filtrate from Tannic Acid precipitation of Albumin

Cow	Mgm. residual nitrogen in 100 gm. milk
Cows on extremely wide (1 : 13) ration	
264	22
293	28
301	29
Cows on extremely narrow (1 : 2) ration	
154	50
292	66
329	51
332	53

The total non-protein nitrogen, amino nitrogen, and urea nitrogen were found to be distinctly higher in the case of cows fed liberally on protein. We have examined the milk of the cows on the 1:2 and 1:13 rations, partly by the methods advocated by Denis and Minot (2) and partly by recognized methods more recently developed, and have obtained the results shown in Table 12. These results substantiate in most respects the findings of Denis and Minot. The amount of total non-protein nitrogen is not in exact agreement with the residual N as determined elsewhere, although the general trend of the results is the same. The agreement is probably as close as could be expected in view of the fact that the samples were taken at different times and that different methods, which involved the use of different protein precipitants, were used.

TABLE 12.—Non-protein Nitrogenous Constituents of Milk at Different Levels of Protein Feeding, Stated as Milligrams of Nitrogen in 100 cc. of Milk

Cow	Total non-protein nitrogen	Urea nitrogen	Amino nitrogen	Creatine and Creatinine nitrogen	Uric acid nitrogen
On low-protein ration					
293	14	3.4	1.23	0.93	0.3
301	11	2.6	1.13	0.83	0.4
264	19	3.6	....	....	....
On high-protein ration					
292	36	21.0	1.99	....	....
329	36	25.0	2.37	1.10	0.3
332	33	19.5	2.75	1.10	0.3

One striking feature of the results shown in Table 12 is that the non-protein nitrogen is not nearly all accounted for by the ingredients which we have determined. The amount of undetermined nitrogen is practically the same for the two groups. This may be in the form of protein-like groups too small to be precipitated along with the proteins but more complex than the amino acids, or it may be present as other unrecognized and undetermined forms. These results show, however, that the outstanding differences between the milk produced at the different levels of protein feeding occurred in case of the urea, the value being about eight times greater in the milk produced on the 1:2 ration than in that produced on the 1:13 ration. The amino nitrogen is also significantly higher in the milk from the high-protein feeding. The nitrogen occurring as creatine and creatinine also appears to be slightly higher on this type of feeding, but there seemed to be no difference in the level of nitrogen occurring as uric acid.

STUDY OF RESULTS ON THE DRY BASIS

Results of chemical analyses are often more evident when considered on the dry or moisture-free basis than when considered as direct percentages of the original material as analyzed. The present results have therefore been studied on the dry basis. From Tables 13 and 14, one might decide at first glance that the proportion of fat in the total solids was distinctly higher and the proportion of lactose correspondingly lower in the case of the high-protein, or 1:4, ration, than of the low-protein, or 1:9, ration. Further study, however—especially an inspection of the data presented in

TABLE 13.—Showing Percentage of Solids Occurring as Various Ingredients on Rations of 1:9 Nutritive Ratio

Cow	Period	Average daily production				
		Average total solids	Average fat	Average protein	Average lactose	Average ash
59	2nd	3.033	0.799	0.748	1.248	.174
	3rd	2.544	0.690	0.618	1.199	.163
	4th	2.119	0.577	0.532	0.913	.128
	5th	2.303	0.626	0.559	0.966	.132
	6th	2.135	0.558	0.554	0.805	.126
111	1st	2.469	0.700	0.595	1.009	.140
	2nd	2.799	0.723	0.704	1.112	.159
	3rd	3.334	0.880	0.826	1.430	.197
	4th	3.861	1.021	0.942	1.609	.230
	5th	3.211	0.906	0.770	1.315	.192
	6th	3.378	0.918	0.823	1.392	.191
154	1st	2.498	0.734	0.616	1.003	.141
	2nd	3.164	0.907	0.775	1.293	.184
	3rd	3.098	0.972	0.746	1.280	.182
	4th	3.836	1.069	0.900	1.636	.214
	5th	3.565	1.046	0.825	1.513	.202
	6th	4.021	1.005	0.990	1.770	.247
146	5th	4.283	1.304	1.086	1.633	.255
Total		55.651	15.435	13.609	23.126	3.257
Percentage distribution of solids			27.73	24.45	41.55	5.85

TABLE 14.—Showing Distribution of the Total Solid Content of Milk Produced on Rations of 1:4 Nutritive Ratio

Cow	Period	Average total solids production	Average fat production	Average protein production	Average lactose production	Average ash production
66	2nd	3.090	0.984	.769	1.188	.171
	3rd	2.346	0.722	.596	0.904	.136
	4th	2.900	0.921	.692	1.112	.163
	5th	3.544	1.071	.865	1.377	.207
	6th	2.506	0.760	.601	0.970	.149
146	1st	3.325	0.999	.827	1.284	.194
	2nd	3.803	1.109	.914	1.516	.226
	3rd	3.570	1.149	.902	1.311	.217
	4th	3.390	1.102	.793	1.289	.191
111	7th	3.678	1.046	.908	1.531	.219
192	1st	2.893	0.872	.738	1.113	.170
	2nd	3.459	1.059	.842	1.345	.186
Total	....	38.504	11.794	9.447	14.940	2.229
Percentage distribution of solids	....	....	30.63	24.53	38.80	5.78

TABLE 15.—Percentage Distribution of Total Solids in Milk Produced by Jersey Cows on Various Rations

Cow	Period	Average daily production				
		Total solids	Fat	Protein	Lactose	Ash
Narrow ration, 1 : 4 nutritive ratio						
53	3rd	3.503	1.292	.894	1.182	.191
53	4th	2.599	0.934	.668	0.804	.134
53	5th	2.759	0.981	.735	0.919	.156
53	6th	2.572	0.925	.695	0.833	.148
53	7th	2.311	0.761	.643	0.707	.134
Total	....	13.744	4.893	3.635	4.445	0.763
Percentage distribution	....	....	35.60	26.44	32.34	5.55
Wide ration, 1 : 9 nutritive ratio						
61	2nd	2.494	0.914	.667	0.764	.119
61	3rd	2.499	0.927	.670	0.780	.123
61	4th	1.561	0.623	.433	0.451	.077
61	5th	2.273	0.853	.615	0.707	.113
Total	....	8.827	3.317	2.385	2.702	0.432
Percentage distribution	....	....	37.57	27.02	30.61	4.89
Medium ration, 1 : 6.5 nutritive ratio						
64	2nd	2.513	0.860	.656	0.832	.129
64	3rd	2.624	0.932	.679	0.804	.135
64	4th	2.720	0.988	.708	0.949	.142
64	5th	2.726	0.985	.690	0.948	.140
64	6th	2.461	0.917	.616	0.825	.124
122	1st	1.449	0.507	.378	0.468	.072
122	2nd	1.615	0.571	.421	0.521	.082
Total	....	16.108	5.760	4.148	5.347	0.824
Percentage distribution	....	....	35.70	25.75	33.19	5.12

Tables 15 to 19, inclusive—will show that this difference is probably due to other causes and has no connection with the level of protein feeding. In Table 15 there is presented the percentage distribution of the total solids for the Jersey cows used in this experiment. Regardless of the ration fed, the fat content of the total solids is invariably several per cent higher and the lactose content correspondingly lower than for the data in Tables 13 and 14. All the data in Table 14, with the exception of one lactation of Cow 111, are from Cows 66, 146, and 192. These cows were all of the same family, and, although they were to most appearances of Holstein breeding, they carried some Jersey blood. With the exception of one lactation by Cow 146, the data in Table 13, on the other hand, are from purebred Holstein cows.

TABLE 16.—Distribution of Solids in Milk Produced by Two Groups on Medium Ration

Cow	Period	Average daily production				
		Total solids	Fat	Protein	Lactose	Ash
Cows having some Jersey blood						
66	1st	3.411	.980	.836	1.384	.191
67	1st	2.734	.821	.673	1.064	.150
67	2nd	2.764	.863	.707	1.000	.155
67	3rd	2.848	.927	.682	1.037	.148
67	4th	2.705	.853	.689	1.039	.151
Total		14.426	4.444	3.587	5.524	0.795
Percentage distribution		....	30.72	24.80	38.19	5.50
Purebred Holstein cows						
59	1st	2.170	.578	.540	0.916	.127
170	1st	2.594	.765	.660	1.025	.151
170	2nd	2.647	.782	.656	1.028	.155
170	3rd	3.351	.985	.812	1.378	.188
59	7th	2.126	.577	.524	0.887	.131
Total		12.888	3.687	3.192	5.234	0.752
Percentage distribution		....	28.60	24.76	40.61	5.83

In Table 16 there are presented separately similar data for the cows fed the 1:6.5 ration. One sub-group comprises one period of Cow 66 and four periods of Cow 67, also a Holstein grade cow carrying some Jersey blood. The other sub-group comprises two periods from Cow 59 and three periods from Cow 170, both purebred Holsteins. The same degree of difference in breeding maintains between these two sub-groups as existed between the cows furnishing the data in Tables 13 and 14. All were on the medium, or 1:6.5, ration. There is seen to be practically the same difference in the distribution of the milk solids between these two sub-groups on the same ration as was shown between the groups on the 1:4 and 1:9 rations in Tables 13 and 14.

In Table 17 there are shown the results from the 1:2, or extremely high-protein ration, and the 1:13, or extremely low-protein, ration, all the cows in both groups being purebred Holsteins. The proportion of the solids occurring as fat is seen to be practically the same for the two groups, which further supports our view that the difference noted in Tables 13 and 14 was due to breed differences and not to feeding. There appears to be some variation with respect to the proportion of protein between the two groups. This variation is not very striking in amount but will be considered along with similar variations of like character in other data.

In Table 18 is shown a similar study of the data from the section of the experiment where the same individual cows received different levels of protein feeding at different times. There will be seen to be comparatively little difference between the proportions of fat in the total solids for the two groups. There is a difference of 1.5 per cent in the proportion of protein in the same direction as that noted in the case of the cows on the 1:2 and 1:13 rations. Although not very striking differences, they are probably worthy of consideration. They seem to confirm the observation of Hills and associates, mentioned in our review, of a slight difference in the proportion of protein in the direction of the feeding.

DISTRIBUTION OF NITROGEN

In view of the conclusion we have previously reached regarding an increase in the amount of non-protein N on the high-protein feeding; and, since this non-protein N is included in the total protein determination, either the total protein (total N x 6.38) must be increased or one of the other nitrogenous constituents must be correspondingly diminished to compensate for the observed increase in this component. The larger percentage of protein observed in the case of the high-protein feeding in Tables 17 and 18 may be considered as fulfilling this condition. The observed increase in proportion of protein accompanying the high-protein feeding in Tables 17 and 18, if converted to its equivalent as per cent of nitrogen in the original milk, would be just about sufficient to account for the increase in amount of non-protein nitrogen as observed in Tables 11 and 12. Table 19 is presented to study the distribution of the various nitrogenous constituents in the groups fed the 1:2 and the 1:13 rations.

TABLE 17.—Percentage Distribution of Solids in Milk Produced on Extreme Protein Rations

Cow	Average daily production					Percentage distribution of solids			
	Total solids	Fat	Protein	Lactose	Ash	Fat	Protein	Lactose	Ash
Extremely low protein rations, nutritive ratio 1:13									
230	2.205	0.594	.528	0.961	.128	26.94	23.95	43.58	5.80
264	1.860	0.542	.453	0.734	.109	29.14	24.35	39.46	5.86
293	1.607	0.452	.433	0.642	.093	28.13	26.94	39.95	5.79
301	2.445	0.731	.616	0.949	.135	29.90	25.19	38.81	5.52
301	2.047	0.586	.484	0.835	.102	28.63	23.64	40.79	4.98
Average						28.55	24.81	40.52	5.54
Extremely high protein rations, nutritive ratio 1:2									
154	2.073	0.558	.564	0.832	.127	26.90	27.20	40.13	6.12
292	3.532	1.100	.932	1.463	.172	31.14	26.39	41.42	4.87
329	3.152	0.837	.836	1.282	.207	26.55	26.52	40.67	6.57
332	2.928	0.827	.707	1.249	.164	28.24	24.14	42.65	5.60
Average						28.21	26.06	41.22	5.79

TABLE 18.—Percentage Distribution of Solids, Same Cows on Different Rations

Cow	Period	Average daily production					Percentage distribution			
		Total solids	Fat	Protein	Lactose	Ash	Fat	Protein	Lactose	Ash
Low-protein periods										
111	Av. 6	3.257	0.881	0.785	1.341	.189	27.04	24.10	41.17	5.80
146	5th	4.547	1.386	1.066	1.735	.271	30.48	23.39	38.07	5.95
154	Av. 4	3.363	0.986	0.813	1.386	.192	29.12	24.17	41.21	5.71
154	Av. 2	3.793	1.026	0.907	1.641	.224	27.04	23.90	43.26	5.90
Average							28.42	23.90	40.93	5.84
High-protein periods										
111	7th	3.761	1.071	0.916	1.566	.222	28.43	24.35	41.61	5.90
146	Av. 4	3.807	1.179	0.938	1.458	.223	30.96	24.63	38.30	5.85
154	Av. 2	2.067	0.557	0.563	0.829	.126	26.91	27.23	40.11	6.10
Average							28.77	25.40	40.00	5.95

TABLE 19.—Distribution of Protein Among the Various Fractions

Cow	Total nitrogen	Casein nitrogen	Albumin nitrogen	Residual nitrogen	Per cent nitrogen as casein	Per cent nitrogen as albumin	Per cent nitrogen as residual
264	.447	.341	.091	.015	76.28	20.35	3.36
230	.443	.341	.072	.030	76.98	16.25	6.77
293	.531	.404	.097	.030	76.08	18.26	5.65
301	.513	.401	.093	.019	78.16	18.13	3.70
301	.490	.393	.089	.008	80.04	18.16	1.63
Total	2.424	1.880	0.442	0.102	387.54	91.15	21.11
Group average	0.485	0.376	0.0884	0.0204	77.51	18.23	4.22
154	.496	.360	.103	.033	72.58	20.73	6.65
292	.488	.352	.098	.038	72.13	20.08	7.78
329	.452	.307	.099	.046	67.90	21.90	10.18
332	.435	.313	.083	.039	71.95	19.08	8.96
Total	1.871	1.332	0.383	0.156	284.56	81.79	33.57
Group average	0.468	0.333	0.096	0.039	71.14	20.45	8.39

The proportion of residual or non-protein N is practically twice as great in the milk of the high-protein-fed cows, in accord with our previous findings. The proportion of the nitrogen occurring as albumin also seems to be slightly higher for this group, although the increased proportion of albumin does not seem to be either very marked or very regular among the different cows. There is seen, however, to be a distinct and regular decrease in the proportion of N occurring as casein in the high-protein-fed cows, as compared with those receiving the ration of opposite extreme. This decrease in proportion of casein for this group is sufficient to counterbalance the increases in both other components. After compensating for the observed increase in albumin, the remaining decrease in casein can be readily accounted for by the diluting effect of the increase of non-protein N, and no specific replacement of casein by non-protein nitrogenous substance is needed to account for the observed differences. A similar study of the data in Table 8 gives similar, though less pronounced, differences between the respective groups of 75.41, 19.90, and 4.64 per cent for the casein, albumin, and residual nitrogen, respectively, for the lower protein feeding and 73.77, 20.12, and 6.71 per cent for these ingredients, respectively, under the higher protein feeding.

ADDITIONAL DATA REGARDING FAT CONTENT OF MILK

Most of the attention of the earlier workers was directed toward determining the effect of different levels of protein feeding on the fat content of the milk, as may be seen by consulting our review of the literature. This is probably true because the fat is the most important ingredient commercially, because the fat is known to be more variable than the other ingredients, and also because simpler and better known methods for the determination of fat were available than was the case with the other constituents. The variety of claims advanced regarding this point seems to entitle it to special consideration at our hands. In addition to the records and analyses made on the days when the milk samples were taken, as described on Page 11, there is available another and independent set of records regarding milk production and fat production. The weight of milk is recorded at each milking and the fat percentage determined on composite samples of milk at regular intervals as a regular procedure for each cow in the Station herd. The records of the cows fed on the 1:4, 1:6.5, and 1:9 rations computed by this system have been carefully studied. They serve only to show that the influence of breed and individuality of the cows was much more potent than the character of the ration in determining the fat content of the milk. In fact, no consistent effect of the level of protein feeding could be traced in these figures; hence, they have been omitted.

There were, however, data from a considerable number of cows in this experiment which have been at various times on widely different rations with respect to protein content. The milk and fat production for these cows for comparable periods on the different rations is assembled in Table 20. One of the most noticeable features among the variations to be found in this table is the relatively high fat percentages which seem to accompany the periods on the herd ration (comparatively low in protein content), which were used as check periods. In most cases, the milk produced in these periods had a higher average test than in any of the experimental periods regardless of the ration under consideration. Several of these check periods, however, represented the first lactation period of the cow or a part thereof, and it has been shown by Monroe (16) that the first lactation period is inclined to run higher in fat content than the succeeding periods. This may be partly, but is probably not wholly, responsible for the difference noted in these periods. The differences may also quite likely be due in part to a tendency of the animal to produce better or more normal amounts of fat on more nearly normal and unrestricted feeding than when held to any arbitrary ration whatsoever.

The figures in this table may lend some support to the view that the very low protein feeding produced a greater decline in fat from the normal level than did feeding rations of the opposite extreme. In fact, we have previously stated in discussing the results of this experiment (49th Annual Report of the Ohio Agricultural Experiment Station) that the low-protein feeding seemed to produce a slightly depressing effect on the fat content of the milk. The present study will show that this effect is by no means uniform, however. It has its important exceptions, where the opposite seems to be true. Considering the great extremes in protein feeding compassed in these experiments and the lack of any progressive or consistent effect produced on the fat content of the milk in either set of data, it seems reasonable to conclude that no important effect on the fat content of the milk produced need be expected from differences in the protein content of dairy rations which are likely to be used in practice. Instances can be selected from Table 20 to support the hypothesis advanced by Taylor and Husband (28) and Tocher (30) that the volume of milk production

TABLE 20.—Average Fat Content of Milk from Same Cows Under Different Conditions of Feeding

Cow	Period	Production		Per cent fat	Nutritive ratio of ration	Notes
		Milk	Fat			
163.........	4th	4,131.2	167.1	4.04	....	Regular herd ration, 5-month period.
	5th	4,532.1	173.6	3.83	1:11	Comparable period with above.
203.........	1st	3,325.5	120.5	3.62	....	Regular herd ration 5 months.
	2nd	3,506.4	118.9	3.39	1:11	Period comparable with above.
203.........	4th	3,004.7	98.5	3.28	1:2	5-month period comparable with others.
293.........	1st	4,379.0	170.8	3.90	....	Regular herd ration, 5-month period.
293.........	2nd	3,446.6	107.4	3.11	1:13	5-month period comparable with above.
293.........	3rd	3,231.9	106.8	3.30	1:13	5-month period comparable with above.
292.........	1st	8,719.0	301.1	3.45	....	Regular herd ration.
292.........	2nd	10,879.0	351.8	3.23	....	Regular herd ration. Roughage grinding experiment.
292.........	3rd	12,139.0	419.8	3.46	1:2	....
292.........	4th	11,369.0	398.8	3.51	1:2	....
301.........	1st	6,948.0	286.5	4.12	....	Regular herd ration.
301.........	3rd	4,608.5	174.4	3.78	1:13	....
301.........	4th	5,301.8	194.4	3.66	1:13	....
301.........	5th	6,555.0	242.9	3.49	1:13	....
146.........	1st	8,377.0	314.3	3.75	1:4	....
146.........	2nd	8,972.0	329.9	3.68	1:4	....
146.........	3rd	9,698.0	376.2	3.88	1:4	....
146.........	4th	10,705.0	441.1	4.12	1:4	....
Total of 4..	....	37,752.0	1461.5	Av. 3.87	....	....
146.........	5th	10,064.0	406.3	4.04	1:9	....
146.........	6th	8,774.0	294.9	3.36	1:11	Aborted at 298 days.
146.........	7th	6,329.0	206.6	3.26	1:11	Period following abortion.
Total of 3..	....	25,167.0	907.8	Av. 3.61	....	....
146.........	8th	10,251.0	368.8	3.59	1:2	....
154.........	1st	6,555.0	248.9	3.80	1:9	....
154.........	2nd	7,456.0	259.1	3.48	1:9	....
154.........	3rd	8,544.0	299.2	3.50	1:9	....
154.........	4th	9,731.0	332.8	3.42	1:9	....
Total of 4..	....	32,286.0	1140.0	Av. 3.53	....	....
154.........	5th	9,644.0	342.0	3.55	1:11	....
154.........	6th	11,793.0	364.6	3.09	1:11	....
Total of 2..	....	21,437.0	706.6	Av. 3.30	....	....
154.........	8th	8,781.0	289.8	3.30	1:2	....
154.........	9th	9,505.0	313.8	3.30	1:2	....

is chiefly instrumental in controlling the fat content of the milk; but, on the other hand, the data from Cow 146 for the first four periods on the same level of protein feeding certainly do not show any evidence of such regulation, for the highest test accompanies the highest volume of milk production in direct opposition to this hypothesis.

COMPOSITION AND PROPERTIES OF THE BUTTERFAT

In Tables 21 to 23, there are presented the results of determinations regarding the composition of the fats. These are presented as weighted lactation averages, as explained on Page 18.

TABLE 21.—Weighted Lactation Average Butterfat Analyses High-protein Ration

Cow	Lactation period	Samples No.	Reichert-Meissl value	Hardness, 10° C.	Iodine value	Saponification value
53	2nd	7	28.8	2.26	34.2	228.6
	3rd	7	28.3	2.03	34.2	229.7
	4th	7	29.0	2.45	33.2	230.3
	5th	8	29.0	2.49	31.9	230.8
	6th	8	28.5	2.00	33.3	235.0
66	2nd	13	26.0	3.01	36.2	223.1
	3rd	6	27.3	2.02	34.9	227.6
	4th	11	28.0	2.69	34.2	228.1
	5th	13	28.1	1.92	34.1	232.7
146	1st	7	26.5	1.83	36.2	231.3
	2nd	4	27.8	1.39	37.0	232.8
	3rd	5	26.3	1.44	36.8	228.2
59	8th	5	27.5	1.40	37.0	231.0
192	1st	6	23.4	1.65	39.8	224.9
	2nd	5	27.8	1.48	40.6	226.6
146	4th	6	27.6	0.91	38.3	228.1
111	7th	7	28.0	1.13	39.9	225.2
Total	....	125	467.9	32.10	611.8	3894.0
Average	....	....	27.5	1.89	36.0	229.1

In the final average figures for the Reichert-Meissl value and saponification value there is remarkable agreement among the groups on the various rations. In the case of the iodine value, the results on the high-protein and the low-protein feeding are not greatly different, but, strangely, the result on the medium-protein ration is considerably lower. The medium-protein ration includes the first experimental periods of all the cows originally placed on the experiment. In most cases, these were not complete lactation periods. Also, there may have been some carry-over effects of the ration previously used affecting these periods. At all events, when these first periods are omitted from the average, the value for the medium-protein group becomes much more nearly like that for the other groups. There is no progressive variation from one extreme of feeding to the other, and the variations between group averages are less than the individual variations within the group; hence, little significance can probably be attached to it.

TABLE 22.—Weighted Lactation Average Butterfat Analyses Medium-protein Ration

Cow	Lactation period	Samples No.	Reichert-Meissl value	Hardness, 10° C.	Iodine value	Saponification value
53	1st	9	29.7	3.69	27.2	232.7
59	1st	5	24.8	2.51	32.7	223.6
61	1st	6	27.0	4.32	27.5	229.7
64	1st	5	27.1	3.70	27.9	229.8
66	1st	9	28.7	3.89	30.1	229.3
67	1st	6	29.9	3.15	30.9	230.2
	2nd	7	29.2	2.97	31.1	230.6
	3rd	6	29.6	1.70	33.8	231.3
	4th	8	28.2	2.49	31.8	232.1
64	2nd	8	27.7	3.16	31.1	230.1
	3rd	6	25.7	2.20	33.4	228.3
	4th	7	27.6	2.86	30.4	230.3
	5th	12	27.0	2.36	32.8	230.4
122	1st	9	26.6	2.24	33.8	231.6
	2nd	8	26.0	1.77	34.7	228.8
157	1st	6	28.2	1.85	31.7	232.8
170	1st	5	24.4	1.15	35.9	228.4
	2nd	5	24.5	1.85	38.3	231.9
	3rd	4	26.5	1.10	39.1	227.6
59	7th	6	26.5	1.65	33.1	236.1
Total	....	137	544.9	50.61	647.3	4605.6
Average	....	....	27.2	2.53	32.4	230.3

TABLE 23.—Weighted Lactation Average Butterfat Analyses Low-protein Ration

Cow	Lactation period	Samples No.	Reichert-Meissl value	Hardness, 10° C.	Iodine value	Saponification value
59	2nd	7	29.3	1.96	32.8	229.7
	3rd	8	28.8	1.14	38.1	228.0
	4th	11	26.3	1.02	37.6	225.4
	5th	8	28.1	1.09	36.9	226.4
61	2nd	13	25.7	3.09	31.1	230.2
	3rd	11	26.1	2.75	30.5	231.5
111	1st	9	26.4	1.08	39.1	225.3
	2nd	9	27.2	1.20	33.9	232.3
	3rd	7	29.4	1.55	33.7	234.3
	4th	4	28.2	0.95	34.1	233.2
	5th	5	27.1	1.76	34.5	235.1
	6th	4	27.5	1.31	36.0	234.2
154	1st	6	25.2	1.90	32.5	232.6
	2nd	4	26.2	1.02	36.7	229.7
	3rd	5	26.7	1.12	35.3	236.2
	4th	3	27.1	1.42	36.4	234.6
146	5th	3	31.1	1.68	33.2	233.9
191	1st	5	27.5	0.61	39.1	227.5
	2nd	5	30.5	0.86	32.9	233.8
Total	....	127	524.4	27.51	664.4	4393.9
Average	....	....	27.6	1.39	35.0	231.3

In Table 24 are shown a few such results available from the 1:2 and 1:13 rations. Here again, the differences between the average results for the group are less for each determination than the individual differences between cows on the same feeding and also less than the differences which may be expected between samples from the same cow on like feeding at different times in lactation. Hence, they may be considered to be of little practical significance.

TABLE 24.—Butterfat Analyses on Extremely High and Extremely Low Protein Rations

Laboratory No.	Cow No.	Date	Reichert-Meissl value	Iodine value	Saponification value	Hardness, 10° C.
Extremely High Protein						
1037	292	10/19/30	29.40	34.67	230.8	1.61
1039	329	10/19/30	32.87	34.71	233.1	1.14
1040	332	10/19/30	28.81	34.25	229.3	1.42
1042	332	12/ 9/31	29.34	34.59	232.1	1.86
1044	329	1/22/32	35.49	33.96	228.9	1.20
1045	332	1/22/32	28.69	38.30	224.7	....
Total	....	....	184.60	210.48	1378.9	7.23
Average	....	....	30.76	35.08	229.8	1.44
Extremely Low Protein						
944	264	8/25/27	26.40	40.60	224.4	0.70
945	301	8/25/27	31.02	33.70	235.4	1.62
966	293	12/ 2/27	24.12	36.76	222.8	0.98
1038	301	10/19/30	30.73	30.67	231.6	2.34
1041	301	12/ 9/31	28.02	30.54	237.1	3.48
1043	301	1/22/32	30.61	29.18	227.9	2.38
Total	....	....	170.90	201.45	1379.2	11.50
Average	....	....	28.48	33.57	229.8	1.91

Some data regarding the hardness of the butterfat are also shown in Tables 21, 22, 23, and 24. The values shown are based on less complete data than was the case with the other determinations, chiefly for the reason that many of the samples of fat which were saved proved too small for this determination.

Considerable differences will be noted between the average values obtained for the groups fed protein at different levels. A study of the data within the different groups, however, will show that the individual variations are greater than those of the group averages. The average value for the medium ration is much higher than that for either the wide or the narrow ration but becomes more nearly like the others by omitting the results of the partial first periods which are included with this group. The hardness data for certain cows which have been at different times on rations of widely different protein content fail to show any marked difference in the hardness of the fat accompanying the change in ration.

Although the data we have presented here are not sufficiently extensive to justify a definite statement regarding the relation of breed to the hardness of butterfat, it is evident that Cows 53, 61, 64, 122, and 157, which were either purebred or high grade Jerseys, produced harder fat than the others in this group. Cows 66, 146, and 192, carrying some Jersey blood, also produced fat which averaged harder than the remaining cows in the list which were of purebred Holstein breeding. This indicates that breed is probably an important factor in determining the hardness of butter. Other unpublished studies of these data indicate also that the stage of lactation exerts considerable influence on the hardness of the fat.

The conclusion seems justified that the protein content of the ration is not the deciding factor in determining the hardness of the fat produced.

SUMMARY

The results of the analyses of several hundred samples of milk, summarized in Tables 4, 5, 6, and 7 according to the protein content of the ration supplied the cows, show very little or no effect of the level of protein feeding on the character of the milk. The only variation which is consistent and progressive from group to group is the percentage of residual or non-protein nitrogen. This ingredient of the milk increased in amount as the level of protein feeding increased.

Other groups of data in Tables 8, 9, and 10 representing the greatest practicable extremes in the level of protein feeding show that the non-protein nitrogen, as mentioned above, is the only observed ingredient whose proportion in the milk is consistently and significantly affected by the level of protein feeding.

The results with respect to the amount of non-protein nitrogen obtained by the usual method of difference have been confirmed by direct determinations of this constituent, as recorded in Tables 11 and 12. The portion of the non-protein nitrogen which shows the greatest amount of variation from change in the level of protein feeding is the urea, which is increased eight-fold from the lowest to the highest group. Amino nitrogen and creatine-creatinine nitrogen are also apparently affected to a lesser extent.

A study of the analyses on the dry basis appears to show that the proportion of protein (total N x 6.38) in the dry matter is increased sufficiently to account for the otherwise observed increase in non-protein nitrogen.

The increase in non-protein nitrogen appears to be coupled with a slight increase in the proportion of albumin on the highest protein feeding. Together these result in lowering, by about 6 per cent, the proportion of total nitrogen which occurs in the form of casein in the milk produced on this ration when compared with the ration of opposite extreme, as shown in Table 12.

A more careful study of the complete data fails to confirm the tentative conclusion previously announced that the low-protein feeding has had a slightly depressing effect on the fat content of the milk.

The character of the fat as indicated by the saponification value, the Reichert-Meissl value, the iodine value, and the hardness has apparently been unaffected by the level of protein feeding.

The great extremes in protein feeding employed in these experiments have produced only relatively minor variations in the composition of the resulting milk. The conclusion seems justified that no changes of major significance in the composition of milk need be expected from the relatively small variations in the level of protein feeding of dairy cows which are likely to be encountered in practice.

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PART II. NUTRITIVE PROPERTIES

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The material presented in the first section of this bulletin is concerned with the chemical and physical properties of milk produced by cows on widely different levels of protein feeding. The data obtained by chemical and physical analyses of milk are of great importance in establishing the value of that product as a food. However, they do not measure certain factors in milk which are now known to be essential for normal nutrition—namely, the vitamins—nor do they take into consideration the biological values of the protein. It became more and more apparent, as the project on high- and low-protein rations continued and the technique for nutritional studies became well developed, that some measurements should be made of the nutritive properties of milk produced on such rations. Consequently, the work here reported was carried out.

It was pointed out in Part I that great differences existed in the nature of the rations fed to the various groups of cows in the later stages of this project. In one group, alfalfa hay was used; in the other, timothy hay. One group received several times as much corn silage as the other, and different feeds were used to make up the grain mixture for each group. Thus, the total amount and the nature of the proteins differed markedly. Possibly, an ideal arrangement would have been to use the same feeds for each group of cows, adjusting the protein level by using purified protein from a single source and the caloric value by means of starch. Such a procedure is possible with rats, but with cows it is difficult because of the large amount of purified material required. Furthermore, it was felt that the use of ordinary feeds would more nearly approach practical conditions.

It has been shown repeatedly that the vitamin content of cow's milk is affected largely by the vitamin content of the ration, except with respect to the vitamin-B complex which the cow seems to manufacture. Any difference in the vitamin content of the feeds making up the rations of the high- and low-protein groups might, therefore, be reflected in the vitamin content of the milk. Similarly, other biological properties of the feeds might exert an influence on the biological properties of the milk.

It can not be justly claimed, therefore, that the data to be presented here constitute a measure of the effect of the level of protein alone. Rather, they must be interpreted in terms of high- and low-protein rations in which the various constituents may exert an influence other than that due to the amount of protein present.

REVIEW OF THE LITERATURE

The investigations concerned with the effect of the cow's ration on the food value of milk are numerous. Good reviews of the literature reporting these studies have been prepared by Randoin (24) and by Maynard (17). Since these reviews were prepared it has been shown that the vitamin-D content of milk can be materially increased by feeding the cow highly concentrated sources of vitamin D, such as irradiated yeast or irradiated ergosterol (14, 27, 30, 31). Recent work has shown that the cow's feed has very little influence on the vitamin-B and vitamin-G content of milk. Hunt and Krauss (11) did find that rapidly-growing pasture grass tended to increase the vitamin-G content of milk, but in later work these same investigators found that feeding cows as much as three-fourths of a pound of such a potent source of B and G as dried yeast exerted practically no influence on the amounts of these factors appearing in the milk.[1]

All the studies referred to above were concerned with the relationship between the amount of certain constituents, such as the vitamins and mineral elements, in the cow's ration and the amounts of these same constituents that appeared in the milk. The writers are not aware of any work having been done with cows to establish a relationship between different levels of protein intake and the value of the milk produced at those levels.

Some work on this problem has been done with rats, and, while an analogy between rats and cows may be questionable, a study of investigations with rats may reveal clues as to what may be expected from cows.

In working with milk secretion in rats the effect of the lactating rat's diet on the milk secreted must be measured in terms of the behavior of nursing young. Thus, McCollum and Simmonds (18) showed that "The quality as well as the quantity of the protein of the diet, together with the content and character of the inorganic portion of the food supply, are factors of importance for milk production commensurate with that of an adequate amount of both the fat-soluble A and water-soluble B".

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[1]Unpublished data.

Hartwell (5) found that large quantities of protein in the diet of lactating rats produced harmful results in the nursing young. The diet consisted of bread and protein in the proportion of 15.0 grams of bread to 5.0 grams of protein. The nurslings grew subnormally and at times exhibited spasms such as those described by McCollum and Simmonds (18) when the mother's diet consisted of 8.2 parts of rolled oats and 18 parts of casein.

In her next study Hartwell (6) used lower levels of protein—15 grams of bread to 1 gram of protein and 15 grams of bread to 2 grams of protein—and used three different sources of protein at each level: casein, edestin, and egg albumin. It was found that in a lactating rat the amount of dietary protein constituting excess, as measured by the weight of the mother and weight and behavior of the young, varies with the type of protein and with the individuality of the rat. The appearance of bad symptoms in the young at the lower level of protein fed led to the conclusion that the rat can metabolize the protein effectively for its own growth but is unable to produce normal milk unless definite quantities of some other constituent or constituents are supplied. The fact that the addition of large quantities of milk or marmite prevented the untoward symptoms in the young led to the belief that some factor, possibly vitamin B (complex), was associated with protein metabolism. In later work (7) Hartwell found that many natural foods or extracts made from them contained a factor which protected suckling rats from the harmful effects of excess protein in the mother's diet and that the distribution of this factor was similar to that of vitamin B (complex). The factor was also found to be similar to vitamin B (complex) in its solubility and its property of adhering to charcoal. Further work showed that a definite, quantitative relationship existed between the amount of protein and the need for vitamin B (complex) in a lactating rat's diet (8) and that the lactating rat, in order to rear her young successfully, must have a greater supply of vitamin B (complex) than at other periods of her existence (9). On an average diet containing 20 per cent protein the lactating rat was found to require three to four times as much vitamin B (complex) as when non-lactating.

In her earlier work Hartwell mentions the possibility of some toxic effect of high-protein diets on the milk secreted. The later work would establish the cause of failure to rear young successfully as lack of vitamin B (complex). Sure (29) attributed infant mortality in rats to inability of the mother to secrete sufficient vitamin B. Daniels and White (3) attributed the greater need for the antineuritic vitamin when high-protein diets were fed to lactating mothers to the increased metabolism resulting from the action of certain amino acids contained in those diets.

Aside from the spasms displayed by the young in McCollum and Simmonds' and Hartwell's experiments, the subnormal growth of the young would indicate limited milk secretion on the part of the mother, as well as failure to secrete a sufficient amount of vitamin B (complex). In the comparison between high- and low-protein diets made by Kozlowska, McKay, and Maynard (12), the differences in growth of the suckling young were probably due entirely to the amount of milk secreted by the mothers.

It is apparent, then, that in the lactating rat the amount of protein fed is of considerable importance in determining the biological value of the milk secreted, as measured by the growth and behavior of suckling young.

In work with the cow, direct measurements of the amount of milk produced and of various nutritive properties of the milk produced on different rations are, of course, entirely possible. The results of some measurements of this kind are herewith given.

EXPERIMENTAL

The milk used throughout this work was obtained from the cows on which some of the work reported in Part I had been done. At the time this phase of the study was begun, the rations of the cows had nutritive ratios of 1:2 and 1:13, respectively. The difference between the rations, therefore, was even greater with respect to protein than in the earlier work in which the extreme nutritive ratios were 1:4 and 1:9. In order to compare the milk from the high- and low-protein groups with that produced by cows receiving a normal ration, three cows receiving the regular herd ration having a nutritive ratio of 1:6.2 were reserved to furnish what is designated in this report as "normal milk". The milk from the cows receiving the 1:2 ration is referred to as "high-protein milk", and that from the cows receiving the 1:13 ration is termed "low-protein milk".

The high-protein ration consisted of alfalfa hay, corn silage in small amount, and a grain mixture comprised of equal parts of linseed oilmeal, cottonseed meal, corn gluten meal, soybean meal, peanut meal, blood meal, wheat gluten, and wheat bran. The low-protein ration was made up of timothy hay, corn silage in large amount, molasses, and a grain mixture consisting of two-thirds corn and equal parts of oats, bran, and starch. (See Page 8 for details). The normal ration consisted of alfalfa hay, corn silage, and a grain mixture made up of 4 parts of corn meal, 3 of oats, 1 of bran, and 1 of linseed oilmeal. The cows were under winter feeding conditions throughout; i. e., they did not have access to pasture.

Owing to the fact that milk was obtained daily from the cows for long periods of time in some of the experiments, the results given for any particular milk do not necessarily represent the production of all the cows in that group. Obviously, when working over long periods of time with cows, various stages of lactation are encountered, as well as occasional dry periods. It is felt, however, that the procedure of obtaining fresh milk daily for the rat feeding experiments allows the results to be interpreted as representative of the production of each group of cows irrespective of the stage of lactation of any individual within a group. It occasionally happened that milk was available from only one cow of a particular group.

Albino rats of our own breeding were used throughout. The mothers of the experimental animals were stock females that had been receiving the regular stock diet of yellow corn meal 67, oilmeal 12, casein 16, alfalfa meal 3, salt 1, calcium carbonate 1, and whole milk *ad libitum.* The litters were reduced to six at birth, and the rats were usually weaned when from 24 to 28 days of age, at which time they weighed from 48 to 52 grams.

PRELIMINARY

It had been observed that at times the feces of the cows receiving the high-protein ration gave off a very bad odor, such as that caused by indol and skatol. This suggested the possibility of some toxic substances being absorbed and secreted in the milk. Consequently, three groups of weanling rats were fed exclusively on milk from the high-protein cows, low-protein cows, and normal cows, respectively. In the first trial of this kind the rats on the high-protein milk died within 4 weeks; whereas those on the low-protein and normal milk survived considerably longer. This suggested some toxicity of the high-protein milk. However, qualitative tests for indol and skatol were negative. Furthermore, in later repetitions of this trial no differences were obtained in the survival period of rats on the three kinds of milk. It was also shown that the cause of death on these exclusive milk diets was nutritional anemia. The earlier post mortem examinations suggested this possibility and later hemoglobin determinations confirmed it.

As soon as it was established that exclusive milk diets resulted in death from nutritional anemia, steps were taken to discover and eliminate the cause. Knowledge of how to supplement milk so as to allow the use of exclusive milk diets would provide a method of procedure whereby the total nutritive effect of any given sample of milk could be ascertained. The work involved in the solution of this problem constituted a study in itself and has been reported elsewhere (13).

VITAMIN A

To determine the vitamin-A content of the milk produced by each group of cows, weanling albino rats were fed a vitamin-A-free diet until no further increase in weight occurred for 3 successive days. Various quantities of milk from each group of cows were then fed daily for 10 weeks, during which time the rats were weighed weekly and observed daily for symptoms of ophthalmia and respiratory trouble.

In preliminary trials, 5, 10, 15, and 20 cc. of each kind of milk were fed daily. It was found that the level which would restore excellent growth after growth had ceased on the A-free diet lay between 5 and 10 cc. for each kind of milk. In these trials several different A-free diets were used, the one finally adopted being made up as follows:

Argentine casein (purified)[2]	18.0
Starch (corn)	66.0
Olive oil (irradiated)	10.0
Salts (Steenbock and Nelson 40)[3]	4.0
Agar	2.0
Dried yeast (Northwestern), 300 mg. per rat daily	
Distilled water, *ad libitum*	

Since the optimum level of milk feeding was found to lie between 5 and 10 cc., levels of 2, 4, 6, and 8 cc. were tried. In this way, any difference between the milks would be emphasized at the lower levels and any screening effect from feeding too much milk would be overcome.

Four rats, two males and two females, were fed on each level of milk. The animals were placed in individual cages at the time supplementary feeding was started and were so distributed that three

[2]Extracted with 95% alcohol for a week and with ether for 24 hours; then air-dried and pulverized.

[3]

Sodium chloride	233.6
Magnesium sulfate	246.0
Disodium phosphate	358.0
Secondary potassium phosphate	696.0
Secondary calcium phosphate	698.0
Calcium lactate	154.0
Ferric citrate	59.8
Potassium iodide	1.6

animals of the same sex out of each litter received the same amount of the respective milks. Eight rats, representing each litter so far as possible, were used as negative controls.

The composite growth curves, representing weekly increments from the time supplementary feeding was begun, are shown in Chart 1. Inasmuch as no satisfactory composite curves can be made of a group of animals in which deaths occur, the data relative to the controls are presented in Table 1.

TABLE 1.—Growth of Rats on Vitamin-A-deficient Diet

Rat	Peak of growth curve	After 1 week	After 2 weeks	After 3 weeks	After 4 weeks	After 5 weeks	After 6 weeks
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
122♂	125	118	122	118	92	Dead	....
123♂	112	112	107	120	94	Dead	....
129♂	123	109	68	Dead	....	....	....
128♂	126	115	88	Dead	....	....	....
124♀	112	115	Dead	....	....	....	....
125♀	121	112	122	118	115	87	Dead
126♀	123	120	96	86	Dead	....	....
127♀	114	112	120	111	80	Dead	....

From the curves in Chart 1 it will be seen that at any of the four levels of milk fed the response from the high-protein or low-protein milk was practically identical. At each level the total increase in weight on the high-protein milk was slightly greater than that on the low-protein milk. This difference was so small, however, as to be of no biological significance and could well have been caused by a greater vitamin-A potency of the alfalfa hay than that obtaining in the timothy hay fed to the low-protein cows. The curves obtained at the 4, 6, and 8 cc. levels of normal milk are practically identical with those obtained with the other two milks at the same levels. At the 2 cc. level the normal milk appears to be decidedly inferior, but in the group of rats receiving this level two severe cases of respiratory trouble prevailed during most of the milk-feeding period.

In addition to comparing the potency of the three kinds of milk, the curves in Chart 1 demonstrate that milk is very potent as a source of vitamin A even under winter feeding conditions and when a not-generally-recommended roughage like timothy hay is fed. Two cubic centimeters of either the high- or low-protein milk gave much better growth than that required by the Sherman system to equal one unit. This is in keeping with the results obtained by MacLeod, Brodie, and Macloon (16) who found that from 0.5 to 0.75 cc. of milk from stall-fed cows allowed growth equivalent to one Sherman unit. Various values for the vitamin-A content of milk have been reported (4, 23, 28), but these values can all probably be associated with the kind and quality of the hay fed to the cows producing that milk.

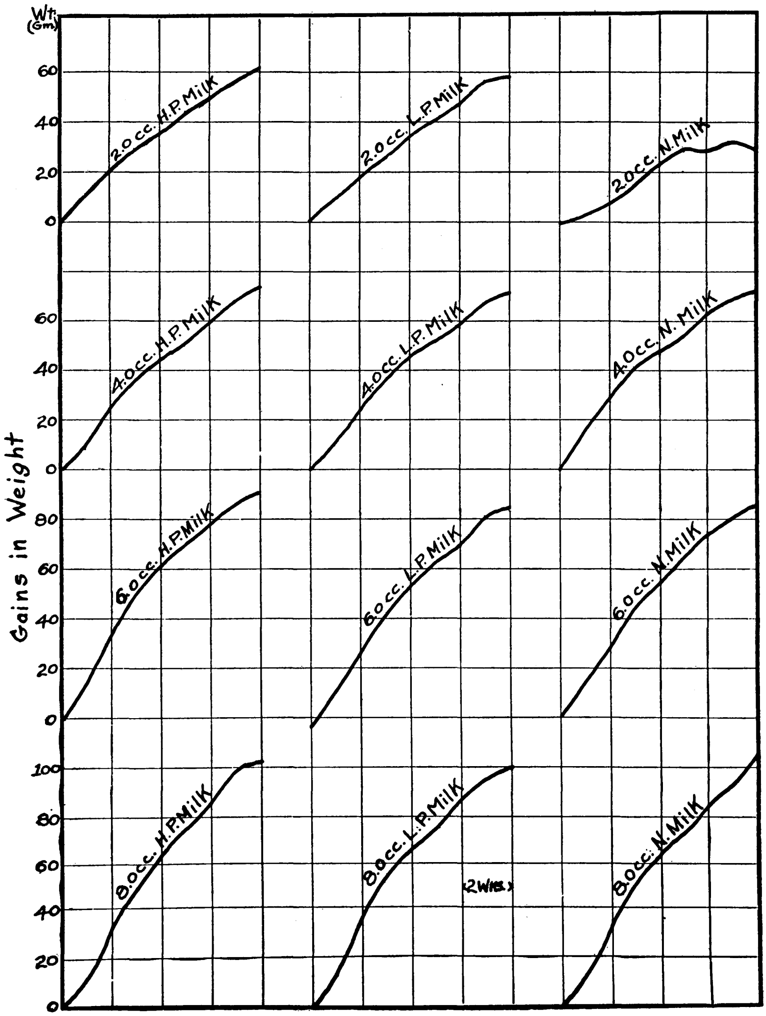

Chart I.—Vitamin-A content of milk from cows on high-protein, low-protein, or normal-protein rations. The curves are plotted as weekly weight increments from the time growth had ceased and supplementary feeding had begun.

At the time the vitamin-A content of the milks reported here was determined the quality of the alfalfa, based on green color, was not as good as that of the timothy. This, together with the fact that the low-protein grain mixture contained a liberal portion of yellow corn, a good source of vitamin A, would tend to overcome the greater vitamin-A potency usually attributed to alfalfa hay.

In general, then, it may be said that the rations of widely differing protein contents exerted very little effect on the vitamin-A potency of the milk produced by the cows fed those rations.

One observation, made during this work and in all succeeding work on vitamin-A-free diets in this laboratory, is worth special mention. In addition to cessation of growth and the development of ophthalmia, more than 50 per cent of the rats on a vitamin-A-free diet develop a paralysis of the hind quarters which sometimes becomes so severe that the animal pulls itself around the cage with its front paws. This condition may be similar to that observed in rats and pigs by Hughes, Auble, and Lienhardt (10), but, unlike the favorable response obtained by these workers with pigs when a source of vitamin A was fed, our rats retain this impaired condition even after growth proceeds and ophthalmia disappears.

VITAMIN B

At the time the assays for vitamin B were made, no distinction had been made between vitamin B and vitamin G. Whenever the term vitamin B is used here, therefore, it refers to what is now known as the B complex.

These assays followed those made on the milk used for the vitamin-A work. A change in the sources of alfalfa hay and timothy hay had been made, the quality of the former being slightly superior to that of the latter.

The details of housing and handling the rats were the same as those followed in the vitamin-A determinations. The basal diet used throughout consisted of:

Argentine casein	19.0
Starch (corn)	63.0
Agar	2.0
Salts (McCollum 185)[4]	4.0
Cod-liver oil	2.0
Crisco	10.0

[4]

Sodium chloride	46.7
Magnesium sulfate, anhydrous	72.0
Monosodium phosphate	93.8
Secondary potassium phosphate	257.7
Primary calcium phosphate	146.0
Ferric citrate	32.0
Calcium lactate	351.8

In preliminary work 5, 10, and 15 cc. of high-protein and low-protein milk were fed after the rats had been for 2 weeks on the B-free diet only. It was found that at least 15.0 cc. of either kind of milk were required for normal growth and that at any of the three levels very little difference could be detected in the response of the animals to either sample of milk. In order to detect any difference between the samples, the milk would need to be fed at smaller level intervals. Consequently, several different levels were included in the study, as follows: 5 cc., 10 cc., 12 cc., 14 cc., 15 cc., 16 cc., 18 cc., and 20 cc. After the preliminary work, the 2-week depletion period was omitted as it was found that very little growth occurred during this time, in most instances the weight remaining constant. The milk-feeding period lasted for 8 weeks in every case. Normal milk was not included in all the assays, but in a sufficient number to establish the critical level and to make a fair comparison with the other two samples. Four rats, two males and two females, comprised a group, except at the 10 and 15 cc. levels where eight rats were used. Eleven rats comprised the control group. In Charts II and III composite growth curves of the various groups are given. The curves for the groups on the 5 cc. level have not been included, inasmuch as all the animals died before the completion of the 8-week period.

It will be seen from a study of Charts II and III that, when 15 cc. of milk were fed, the rats receiving the high-protein milk responded better than did those receiving the low-protein milk. It may immediately be suggested that this difference could be attributed to a greater amount of vitamin B in the alfalfa hay. The work of Bechdel and Honeywell (1), in which it was shown that a cow receiving a ration lacking in vitamin B was capable of synthesizing this vitamin and secreting it in her milk, would preclude this possibility. Furthermore, unpublished work of Krauss and Hunt (15) had shown that, when the cow's intake of both vitamins B (B_1) and G is greatly increased, no corresponding increase occurs in the amounts of these two factors found in her milk. Also, reference to the charts shows that the normal milk was richer in vitamin B than was either the high-protein or low-protein milk. The cows in the normal group received hay from the same source as that fed to the high-protein cows. This would suggest the possibility of a relationship between protein intake and secretion of vitamin B in the milk. At the normal level of protein feeding, the amount of vitamin B secreted in the milk was maximum; at the low-protein level the amount of vitamin B secreted in the milk was appreciably reduced;

whereas at the high-protein level the reduction in vitamin-B content of the milk was not so great. It required 15 cc. of normal milk, 16 cc. of high-protein milk, and 20 cc. of low-protein milk to allow good, uninterrupted growth for 8 weeks in rats receiving a diet lacking in vitamin B but otherwise complete.

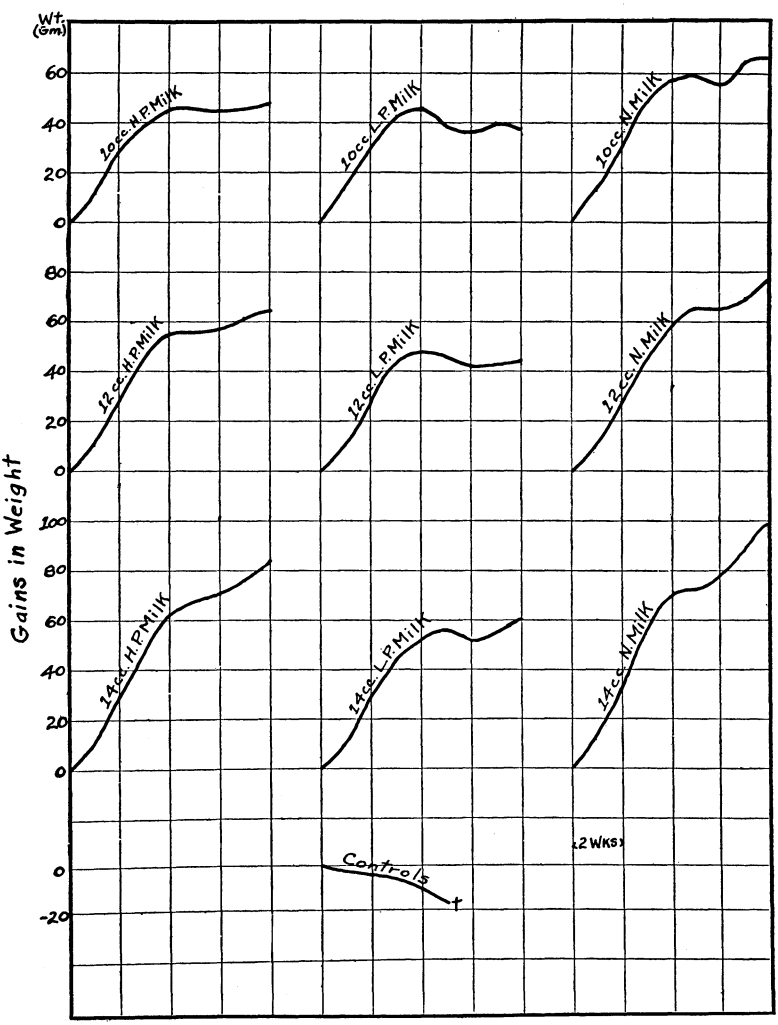

Chart II.—Vitamin-B (complex) content of milk from cows on high-protein, low-protein, or normal-protein rations. The curves are plotted as weekly weight increments

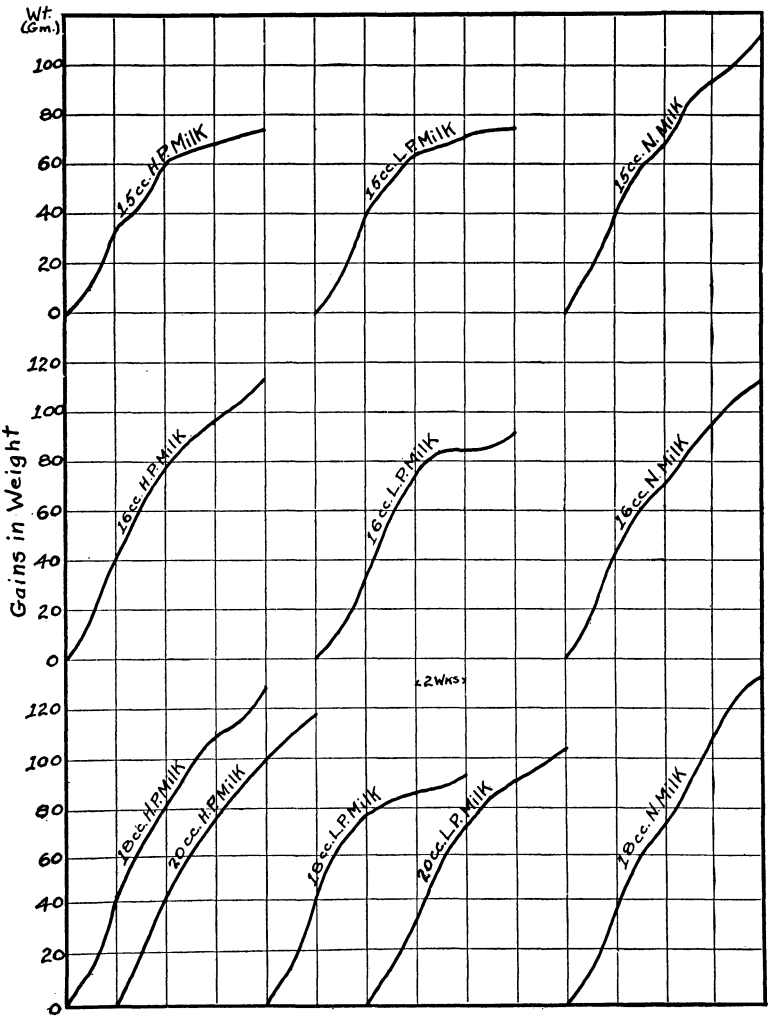

Chart III.—Same as Chart II but at higher levels of milk feeding

VITAMIN D

As the first step in determining the vitamin-D content of the milk produced by the three groups of cows used in this experiment, 10 cc. of each kind of milk were fed daily to rats receiving the Steenbock and Black rickets-producing diet of 76 parts of yellow corn, 20 of wheat gluten, 3 of calcium carbonate, and 1 of salt. One group of rats was killed at the beginning of the experiment to serve as a check on the ash content of the bones of rats when started on experiment. Another group received the rickets-producing diet only. Since rickets is associated with bone formation, the ash content of the femurs of the rats after 4 weeks on experiment could be used as a preliminary measure of the calcifying properties of the different samples of milk.

At the end of 4 weeks the rats were killed and their femurs removed. After freeing the bones from adhering tissue they were dried in a steam oven for 24 hours. They were then crushed, wrapped in filter paper, placed in a Soxhlet extraction tube, and extracted with 95% alcohol for 48 hours and with ether for 24 hours. After allowing any excess of ether to volatilize, the bones were placed in a constant temperature oven at 100° C. until they became constant in weight. Following this they were ashed in an electric muffle furnace and the amount of ash present determined. The results given for ash in Table 2, therefore, are on a moisture-free, fat-free basis. As shown in Table 2, 10 cc. of any sample of milk did not constitute a sufficiently large dose to maintain the ash content of the femurs at the value found for them at the time the rats were started on experiment. The low-protein and normal milks gave similar ash values, both of which were higher than the value obtained with the high-protein milk.

TABLE 2.—The Relative Vitamin-D Content of Milk from High-protein, Low-protein, and Normally Fed Cows, as Indicated by the Percentage of Ash in Rat Bones

Group	Rats	Ash in femurs	Initial weight	Final weight
	No.	*Pct.*	*Gm.*	*Gm.*
Check, killed at beginning	5	46.28	50	....
Basal ration only	2	33.05	50	57
Basal ration plus 10 cc. milk from high-protein cows	4	41.63	45	84
Basal ration plus 10 cc. milk from low-protein cows	4	45.89	48	93
Basal ration plus 10 cc. milk from normal cows	4	44.24	47	82

The addition of milk to a rickets-producing diet alters the calcium-phosphorus ratio. Outhouse, Macy, and Brekke (22) overcame this difficulty by adjusting the Ca:P ratio accordingly. Another method for determining the vitamin-D content of milk consists of obtaining the pure fat from the original milk samples and calculating the potency of the milk from the vitamin-D value obtained for the fat.

Based upon the calcifying values shown in Table 2, pure fat samples, obtained by the method described in Part I, were fed at 400 and 800 mg. levels, using the same procedure followed with the liquid milk samples. The results obtained in this trial are shown in

Table 3. At either the 400 mg. or 800 mg. levels the low-protein milk fat gave higher bone ash values than did either of the other two fats.

TABLE 3.—The Relative Vitamin-D Content of Butterfat from High-protein, Low-protein, and Normally Fed Cows, as Indicated by the Percentage of Ash in Rat Bones

Group	Rats	Ash in femurs	Initial weight	Final weight
	No.	*Pct.*	*Gm.*	*Gm.*
Check, killed at beginning of experiment	4	43.65	49	....
Basal ration only	5	27.61	48	79
Basal ration plus 0.4 gm. high-protein fat	4	41.16	48	78
Basal ration plus 0.8 gm. high-protein fat	4	47.23	46	74
Basal ration plus 0.4 gm. low-protein fat	5	44.10	48	80
Basal ration plus 0.8 gm. low-protein fat	4	48.50	48	76
Basal ration plus 0.4 gm. normal fat	4	38.94	48	80
Basal ration plus 0.8 gm. normal fat	4	42.59	48	86
Basal ration plus 0.2 gm. cod-liver oil	5	48.42	50	84

The results obtained by the prophylactic procedure just described were substantiated by another trial in which the standard curative technique was followed (Table 4).

Rats were fed the Steenbock and Black diet for 24 days, at the end of which time they had developed severe rickets. At this point 400 mg. and 800 mg. of the different fat samples were added daily for 10 days. The fat was melted over a low flame and measured out with a standardized pipette into individual glass dishes. At the end of 10 days the rats were killed. The radii and ulnae were removed and placed in 10% formalin. After not less than 4 hours these bones were examined for calcification according to the line test of McCollum et al. (19).

The data in Table 4 show that the low-protein fat contained the most vitamin D, followed in order by the high-protein fat and the normal fat. Thus, the data obtained by the curative procedure fully substantiated those obtained prophylactically.

Inasmuch as the vitamin-D content of grain is negligible, the difference in vitamin-D potency found in these samples of fat may have been due to the kind of hay fed or to the silage, since different amounts were fed to each group. Samples of the alfalfa hay and timothy hay fed to the cows at the time the fat samples were obtained were tested for vitamin-D potency. The timothy hay was found to be slightly superior in this respect.

TABLE 4.—The Relative Vitamin-D Content of Butterfat from High-protein, Low-protein, and Normally Fed Cows, as Indicated by the "Line Test"

Supplement to rickets-producing ration	Rat No.	Weight	Average daily food consumption	Calcium* deposition
		Gm.	*Gm.*	
Butterfat from high-protein cows, 0.4 gram...	635	57—70	6.5	—
	645	61—70	7.7	—
	652	75—88	9.0	—
	684	65—80	11.3	—
	693	64—74	7.6	—
Butterfat from high-protein cows, 0.8 gram...	640	65—79	12.0	++
	648	70—84	9.0	+
	655	60—77	7.6	+
	692	69—80	9.1	+
	697	80—88	8.5	+
Butterfat from low-protein cows, 0.4 gram	636	73—90	9.7	—
	646	64—74	10.1	+
	653	62—76	7.7	+
	685	62—77	10.9	+
	694	70—79	8.5	—
Butterfat from low-protein cows, 0.8 gram	641	66—73	7.5	++
	649	65—73	7.8	++
	656	60—74	11.5	+++
	683	66—86	12.5	+++
	689	65—80	11.3	++
Butterfat from normal cows, 0.4 gram	637	64—78	9.4	—
	647	65—75	9.0	—
	654	66—71	7.4	—
	686	62—82	9.9	—
	696	62—72	7.4	—
Butterfat from normal cows, 0.8 gram	642	70—76	6.0	—
	650	64—71	7.0	+
	657	57—72	9.0	+
	690	70—86	9.0	+
None	638	67—80	9.5	—
	643	73—78	7.5	—
	651	70—76	8.0	—
	688	64—70	10.0	—
	691	69—68	7.5	—
0.2 gram cod-liver oil	639	68—76	8.9	+++
	644	78—82	9.2	+++
	687	52—63	8.5	+++

*No calcium deposition (—); calcium deposition begun (+); moderate calcium deposition (++); advanced calcium deposition (+++); complete calcium deposition (++++).

Another difference in the feeding program consisted in the fact that the low-protein cows received some molasses daily. However, when biological assays were made on a sample of the molasses fed, no vitamin D was found. It would seem, therefore, that the difference in vitamin-D content of the hays was at least partly responsible for the differences found in the vitamin-D potency of the fats. Since the vitamin-D content of the silage was not determined and since no published data on the potency of corn silage in this factor are known, the effect of this roughage on the vitamin-D content of the fat cannot be estimated.

While the differences in vitamin-D content observed were uniform in the several trials, they were not great enough to suggest the possibility that a relationship existed between the level of protein fed to cows and the ability of those cows to secrete vitamin D into their milk. The data obtained do show that cow's milk is a relatively poor source of the antirachitic factor, at least 23 cc. of milk (800 mg. of butterfat) being required daily to allow practically normal bone formation in rats fed a rickets-producing diet.

TOTAL NUTRITIVE EFFECT

The ideal method of comparing two foods would consist of feeding each of two groups of animals on one of the foods exclusively over a sufficient length of time to measure the effect upon all the life processes. Owing to nutritive deficiencies that exist in practically every food, including milk, such an ideal can never be realized. When milk constitutes the sole source of diet for rats nutritional anemia, resulting eventually in death, occurs. However, when small amounts of copper and iron are added to milk, the onset of anemia is prevented and growth proceeds at a fairly good rate. Knowledge of this fact led to the following procedure which was intended to show the total nutritive effect of the milk from each of the three groups of cows.

Albino rats were taken at weaning age (24 days) when they weighed about 50 grams and were divided into three groups of eight each. One group was given free access to milk from the high-protein cows, a second group to milk from the low-protein cows, and the third group to milk from the normal-protein cows. The rats were fed as individuals twice daily, 0.5 mg. of iron and 0.16 mg. of copper being added to the afternoon allowance of milk. A careful record was kept of the milk consumed. The animals were weighed once a week and were continued on this program for 12 weeks. The results obtained are recorded in Table 5, from which it will be seen that both the females and males receiving the low-protein milk gained better than did those receiving either of the other two milks. When calculated to the basis of gain per 100 cc. of milk consumed, the results still favor the group receiving the low-protein milk. Inasmuch as the sex distribution in the high-protein and low-protein groups was equal, the data for males and females could be combined and treated statistically. Application of Student's method showed that the greater numerical gain of the rats in the low-protein group was biometrically insignificant.

TABLE 5.—Comparison of Rates of Gain of Rats Fed Milk Exclusively (Plus Copper and Iron) from High-protein, Low-protein, and Normal Cows

Group	Number and sex of rats	Gain in weight (12 weeks)	Total milk consumed	Gain per 100 cc. milk
		Gm.	*Cc.*	*Gm.*
High-protein	3♀s	77	3684	2.09
	5♂s	96	3810	2.52
Low-protein	3♀s	98	3843	2.55
	5♂s	110	3846	2.86
Normal	4♀s	87	3850	2.26
	4♂s	96	3840	2.50

BIOLOGICAL VALUE

Although this trial may be looked upon as measuring the total nutritive effect of the different milks, it must be pointed out that the consumption of milk was great enough to insure adequate intake of energy, the necessary vitamins, and minerals. In a sense, then, this trial was a measure of the efficiency of the proteins of the milk.

It was felt, therefore, that the slight numerical increase in growth obtained on the low-protein milk in the previous trial might be emphasized by using synthetic diets to which was added, as the sole source of protein, skimmilk powder. Consequently, samples of skimmilk powder were prepared from milk obtained from each of the three groups of cows. The milk was dried in shallow, enamel trays in a home-made dryer, heated with steam coils. Air circulation was facilitated by the use of large electric fans. The temperature of the drying chamber never exceeded 50° C.

The samples of milk powder were analyzed for nitrogen, and, based on these analyses, a sufficient quantity of the powder was added to a protein-free basal diet to furnish 9 per cent of protein, as indicated in Table 6. Molasses, rather than yeast, was used as a source of the B complex in order to avoid the introduction of another source of protein. Each of four groups of albino rats was allowed free access to one of these diets. The animals were fed as individuals for 12 weeks and a daily record of food consumption was kept. The *ad libitum* system of feeding was followed since the results were to be interpreted in terms of gain per gram of protein intake, as proposed by Osborne, Mendel, and Ferry (21). The 9 per cent level of protein was chosen in order to allow growth somewhat slower than normal and thus avoid any screening effect due to waste, as occurs when excessive protein is fed.

TABLE 6.—Composition of Diets Used to Compare Protein Efficiencies of Skimmilk Powders

Basal		Basal plus skimmilk powder from high-protein cows	Basal plus skimmilk powder from low-protein cows	Basal plus skimmilk powder from normal cows
Cod-liver oil	2.00	2.00	2.00	2.00
Salts (185)	4.00	4.00	4.00	4.00
Crisco	10.00	10.00	10.00	10.00
Molasses	7.50	7.50	7.50	7.50
Dextrin	76.50	51.08	47.41	50.90
Skimmilk powder	......	25.42	29.09	25.60
Total protein (%)	0.406	8.80	8.97	9.09

In this trial both sexes were used. The results obtained are therefore presented for each sex in Table 7. Those rats receiving the protein-free basal diet lost weight rapidly and died within 4 weeks.

It is apparent from Table 7 that no statistical treatment is necessary to show that no significant differences in gain per gram of protein intake were obtained, although this was done as routine procedure.

TABLE 7.—Gain per Gram of Protein Intake. Skimmilk Powders from High-protein, Low-protein, and Normal Cows Used as Sources of Protein

Source of skimmilk powder	Sex of rats	Initial weight	Final weight	Gain in weight	Food intake	Protein intake	Gain per gm. of protein intake
		Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
Milk from high-protein cows	♀	52	203	150	915.1	80.45	1.86
	♂	54	236	182	966.9	84.95	2.14
Milk from low-protein cows	♀	52	188	135	855.1	78.09	1.74
	♂	53	245	193	931.3	82.91	2.32
Milk from normal cows	♀	46	181	135	859.7	78.07	1.73
	♂	54	237	182	954.5	85.68	2.14

It was not to be expected that the protein of milk would undergo any change under any system of feeding cows. Proteins are definite chemical substances with certain amino acid combinations. Any ration lacking in the necessary amino acids for milk formation, aside from those elaborated in the body, might be expected to result in diminished milk flow rather than the production of milk in which the efficiency of the protein would be altered. However, it was not felt that the data obtained in the trial just described were sufficient to prove this assertion absolutely. Consequently, a further experiment was carried out, using the paired feeding method of Mitchell and Beadles (20) and limiting the comparison to the high-protein and low-protein milks.

Samples of skimmilk powder were prepared as previously described. The sample obtained from the high-protein cows was found to contain 33.3 per cent protein; that from the low-protein cows, 32.35 per cent protein. Preliminary trials in which skimmilk powder furnished protein to the extent of 5, 7, and 9 per cent of the diet, respectively, showed that the 9 per cent level was necessary to secure good, but slightly subnormal, growth. The following diets were compounded:

Cod-liver oil	2.0	2.0
Salts (185)	4.0	4.0
Crisco	10.0	10.0
Skimmilk powder	27.0[5]	27.8[6]
Dextrin	57.0	56.2

In addition, each rat received daily as a source of the B complex 250 mg. of Northwestern dried yeast.

In order to insure that no loss of potency of the cod-liver oil would occur, each of the diets was mixed on several different occasions, both diets being mixed on the same day. The protein content of each batch was determined, and from this an average value for each diet was calculated. The diet containing the high-protein skimmilk powder averaged 9.37 per cent protein; that containing the low-protein skimmilk powder, 9.25 per cent protein. These values are higher than the calculated objective (9 per cent) and were found subsequently to be due to the nitrogen carried by the dextrin.

Eleven pairs of male albino rats were fed over a period of 70 days. The results obtained are tabulated in Table 8.

Theoretically, if the technique involved in carrying out the paired feeding method is done flawlessly, the food consumption of each rat would be identical with that of its pair mate. When such is the case or when this condition is approximated, the results may be interpreted in terms of gain in weight. The method suggested by Mitchell and Beadles (20) was applied to the weight gains shown in Table 8. These gains represent the total result of eight observations for each of nine pairs and nine observations for each of two pairs, making a total of 90 comparisons. Of these, 48.5 favored the rats receiving skimmilk powder from the low-protein cows. This is a deviation of 3.5 from the mean value, 45, which would obtain were the results due to chance 50 per cent of the time. The standard deviation of the frequency distribution of 90 comparisons

[5]Prepared from milk from high-protein cows.
[6]Prepared from milk from low-protein cows.

($\sqrt{0.5 \times 0.5 \times 90}$) is 4.74. This is greater than the actual difference, 3.5, and means, therefore, that the differences are entirely due to chance.

TABLE 8.—Efficiency of Proteins of Skimmilk Powder, as Measured by Paired Feeding Method (Based on Gain per Gram of Protein Intake)

Pair number	Initial weight	Final weight	Gain in weight	Food intake	Protein intake	Gain per Gm. of protein intake
	Gm.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
1	50 56	232 245	182 189	865.4 840.3	81.09 77.73	2.24 2.43
2	52 48	233 248	181 200	815.4 830.6	76.40 76.83	2.37 2.60
3	54 55	234 232	180 177	776.1 791.1	72.72 73.18	2.48 2.42
4	48 50	186 196	138 146	632.7 666.0	59.58 61.61	2.32 2.37
5	60 59	216 252	156 193	813.1 817.8	76.19 75.65	2.05 2.55
6	54 57	204 208	150 151	670.5 653.7	62.83 60.47	2.39 2.50
7	50 53	198 176	148 123	599.0 572.4	56.13 52.95	2.64 2.32
8	54 54	196 190	142 136	625.1 618.5	58.57 57.21	2.42 2.38
9	54 53	206 214	152 161	687.4 695.7	64.40 64.35	2.36 2.50
10	52 50	184 196	132 146	601.8 639.7	56.38 59.17	2.34 2.47
11	54 56	218 228	164 172	746.9 768.5	69.98 71.09	2.34 2.42

Application of Crampton's statistical method for paired feeding data (2) to the above also showed the results to be without significance. The necessary difference to demonstrate significance was 9.84 grams; whereas the mean difference was only 6.27 grams.

Since considerable variation existed in the food consumption of individuals of some pairs, it was felt that a more accurate treatment of the data could be made on a basis of gain per gram of protein intake. Application of Crampton's method to the last column of figures in Table 8 shows the data to be statistically insignificant.

It has been established beyond doubt, therefore, that no difference existed in the growth-promoting properties of the proteins contained in the two samples of skimmilk powder. These results obtained by the biological method are in keeping with the results obtained by the usual methods of chemical analysis. The differences shown by micro methods with respect to the nitrogenous constituents (Part I) are apparently without biological effect.

VALUE FOR CALVES

The experiments thus far reported are essentially fundamental in nature, using the usual biological procedure with small animals for detecting any differences in certain nutritional factors. No matter what the outcome of these experiments may have been, it was felt from the beginning that a practical feeding trial of some sort would be needed to help answer the questions involved. Consequently, calves were chosen as the experimental animals.

Female calves, dropped by cows in the Experiment Station herd, were used. As each animal was born it was assigned to one of three groups. This was continued until there were five Holstein calves and one Jersey calf in each group. The general plan was to feed whole milk, hay, and grain in the usual way, according to a definite schedule. Milk was fed according to the size of the calf and was limited to 16 pounds a day. Alfalfa hay was fed *ad libitum.* A grain mixture, consisting of 300 parts of corn, 100 of wheat bran, and 100 of linseed oilmeal, was fed according to the ability of the calf to consume it. Group I received whole milk from the cows receiving the high-protein ration; Group II, whole milk from the cows receiving the low-protein ration; and Group III, whole milk from the cows receiving a ration normal with respect to protein.

For 3 days after birth the calves were allowed to nurse. They were then moved to individual calf pens and fed warm, whole milk twice a day. Hay and grain feeding usually started when the calves were between 3 and 4 weeks old. The calves were weighed and measured weekly during the 6 months' trial.

The results obtained in this experiment are summarized in Table 9. It will be observed that the figures for Groups I and II are very similar, showing that, without question, in this type of feeding trial milk from either the high-protein or low-protein group of cows could be used with equal effect. The gains made by the calves in Group III are numerically slightly greater on all bases of calculation than those for the calves in either Group I or Group II. Owing to the number of animals in a group and the considerable variation shown by individuals, these slight differences are not biologically significant.

Except for slight cases of scours, such as are commonly found among calves, all the animals showed a fine health record, and, from physical appearances, were in excellent condition at all times.

From the standpoint of calf-raising, therefore, it is apparent that cows may be fed extremes of protein without affecting the value of their milk.

TABLE 9.—Comparison of Milk from High-protein, Low-protein, and Normal-protein Cows for Practical Calf Feeding

	Group I High-protein milk	Group II Low-protein milk	Group III Normal milk
No. of calves*	6	6	6
Initial weight, lb.	88.7	80.5	83.5
Final weight, lb.	373	367	391
Gain in weight, lb.	284	286	307
Av. daily gain, lb.	1.56	1.59	1.67
Initial height, in.	29.71	28.88	29.13
Final height, in.	40.93	40.46	40.93
Gain in height, in.	11.23	11.60	11.81
Food consumption, lb.:			
Whole milk	2406	2427	2347
Hay	344.8	383.2	357.2
Grain	247.8	198.7	237.0
Dry matter	839.5	834.3	833.8
Gain per 100 lb. milk:			
Weight, lb.	11.80	11.78	13.08
Height, in.	0.467	0.478	0.503
Gain per 100 lb. dry matter:			
Weight, lb.	33.83	34.28	36.82
Height, in.	1.34	1.39	1.42

*Five Holstein females and 1 Jersey female in each group.

DISCUSSION

In general, the results obtained in all the experiments reported in Part II were negative. While all the possible comparisons were not made, enough of the essential factors were studied to indicate that further delving would probably prove fruitless.

After the B complex was separated into two generally accepted entities, B and G, some consideration was given to assays of the high-protein, low-protein, and normal milks for these factors. However, the work of Hunt and Krauss (11) and Krauss and Hunt (15) showed that the vitamin-B content of milk was unaffected by the cow's ration and that the vitamin-G content was but slightly affected.

Studies on vitamin C were not conducted because it had been shown that cows apparently belong to that group of animals having no specific requirement for vitamin C. Any change in the vitamin-C content of milk produced on different rations could, therefore, be traced directly to the vitamin-C content of the rations. This holds true to some extent for vitamins A and D, although it has been shown that bovines have a definite requirement for these vitamins. The possibility of a sparing action, or vice versa, traceable to the amount of protein fed, was therefore a possibility worthy of consideration.

Slonaker (25), working with rats and using synthetic diets, found that the rate of gain of young from mothers receiving varying amounts of protein was least at the lowest level of protein fed (10 per cent) and in general increased with each addition of protein, up to 26 per cent. This would indicate a definite effect of protein level on milk quality were it not for the fact that the greatest mortality occurred in the offspring of the group receiving the highest percentage of protein and that the number of young per litter varied.

In another paper Slonaker (26) showed that the loss of weight of the mothers while nursing was greatest in the group receiving 10 per cent of protein and became less as the per cent of protein in the diet increased. This corresponds with the response of the cows on the low-protein ration. These animals freshened in good condition, but as lactation proceeded they lost flesh rapidly. The cows on the high-protein ration did not show this loss. Slonaker's data also show that the quantity and quality of milk secretion in rats were poorest in the group receiving 10 per cent protein and increased as the protein content of the diet increased. Our low-protein cows, likewise, produced less milk than did the high-protein cows, and, as has been demonstrated in the experiments reported, a slight difference in quality was obtained with respect to the vitamin-B complex.

SUMMARY

The food values of milk produced by cows fed extremely high-protein, extremely low-protein, or normal-protein rations were compared. Vitamins A, B (complex), and D, total nutritive value, and biological value of the proteins were included in the studies made with rats. A practical feeding trial with heifer calves was also conducted.

Slight differences were found in the vitamin-A and vitamin-D contents of the three kinds of milk. These differences could be traced to the vitamin-A and vitamin-D contents of the rations.

Enough difference was found in the vitamin-B (complex) content of the milks to indicate that some relationship may exist between the level of protein feeding and the amount of vitamin B (complex) secreted in the milk. Fifteen cc. of "normal milk", 16 cc. of "high-protein milk", and 20 cc. of "low-protein milk" were required to allow normal growth on a basal diet deficient in the B complex.

No difference was found in the total nutritive effect, based on the method of exclusive milk feeding, or in the biological value of the milk proteins, based on gain per gram of protein intake.

No significant difference in rates of gain was obtained with calves raised in a practical way on milk from any of the three sources.

CONCLUSION

Cows may be fed rations varying markedly with respect to protein quantity without materially influencing the biological value of the milk produced on those rations. In spite of the great variety of proteins in the high-protein ration and the meager variety in the low-protein ration, the resulting milk remained remarkably constant in its biological value.

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